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EFFECT OF ALPHA-NAPHTHALENE ACETIC ACID ON THE RATE OF GERMINATION OF SEEDS

I. Leguminous Seeds

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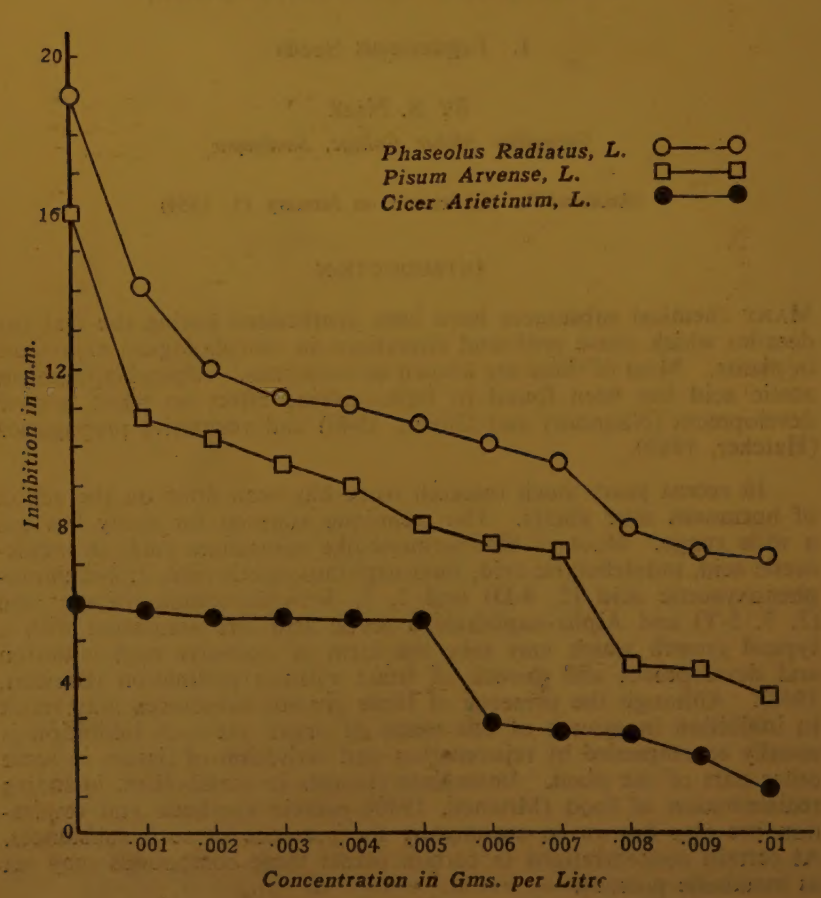
Introduction

Many chemical substances have been synthesized during the last two decades which cause profound alterations in morphological expression in plants. Most of these are known as hormones. Alpha-Naphthalene acetic acid has been found to have striking effect on plant growth development (Naundorf and Oliver, 1949) and vegetative propagation (Hatcher, 1949).

In recent years much research work has been done on the action of hormones over plants. The technique adopted for study has got a wide range. Most of the hormone-like substances such as indole-acetic acid, indolebutyric acid, Beta-naphthoxyacetic acid, 2, 4-dichloro-phenoxyacetic acid (2, 4-D) and 2, 3, 5-trichlorophenoxyacetic acid (2, 3, 5-T) and Alpha-naphthalene acetic acid are associated with a typical growth which may take the form of excessive root initiation and development and growth of fruits without pollination (Brevieri, 1948). Although the presence of these growth substances may result in inhibition in growth of one tissue or organ yet such inhibition is usually accompanied by rejuvenation and activation of tissues in some other part of the plant. Immediate changes in metabolism, including redistribution of food (Mitchell, 1940), protein synthesis and respiration are also known to accompany the presence of such substances. At certain concentrations in certain plants these compounds may act as metabolic poisons.

Material and Methods

Five healthy and fresh seeds of each of the species were selected for experiment. The seeds were soaked in watery solution of Alpha-naphthalene acetic acid taken in different small beakers at various concentrations ranging from 1 p.p.m. to 10 p.p.m. Distilled water was used for soaking the controls. The duration of soaking the seeds was 6, 12 and 18 hours respectively. The seeds after treatment with Alpha-naphthalene acetic acid were thoroughly washed with tap water. Blotting-papers were taken in trays and were moistened with tap water. The trays were then placed in germinating chambers under controlled temperature (30° C.) and humidity (87%). The seeds were germinated on moist filter-papers at 30° C. which was very near the atmospheric temperature. The elongation of the radicles of the five seeds were

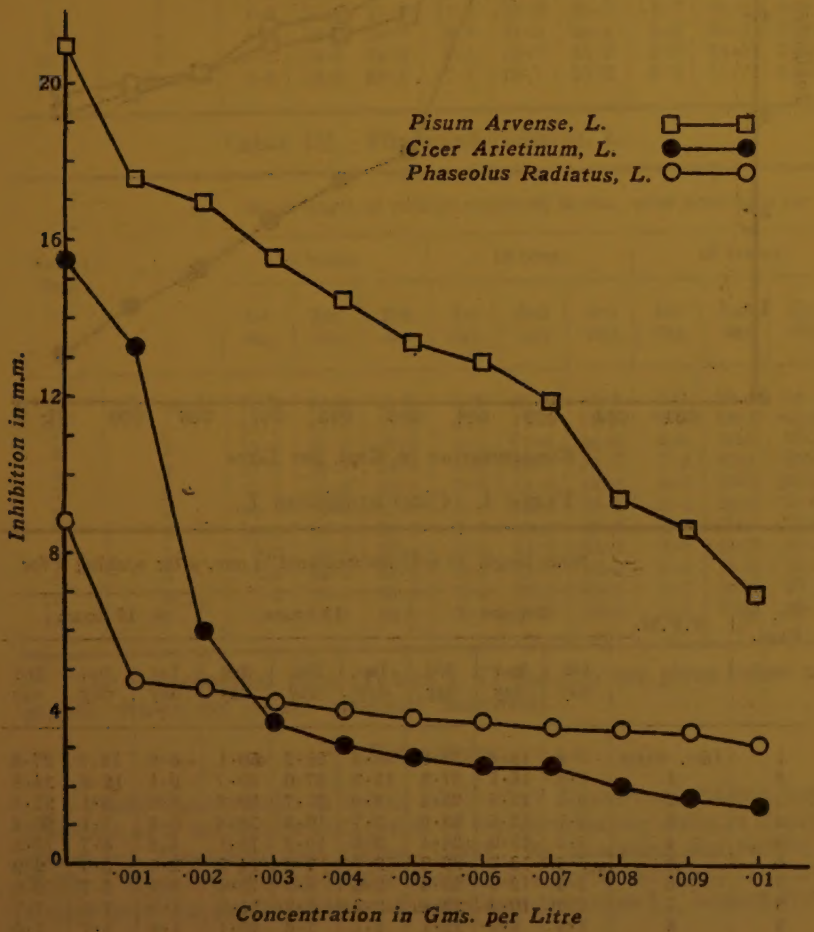


measured and the mean reading was recorded. The measurements were taken with the help of threads and a scale.

EXPERIMENTATION

Dykyj and Dykyj (1943) suggested that when wheat kernels were soaked for 24 hours in solutions of Alpha-Naphthalene acetic acid in different concentrations at 25° C. the mean germination time was increased proportionally to the concentration. Since Alpha-naphthalene acetic acid proved effective suppression of germination energy in single substance tests were carried on three different seeds to determine if Alpha-naphthalene acetic acid is differentially effective.

12 HOURS SOAKING. 1st DAY READING.



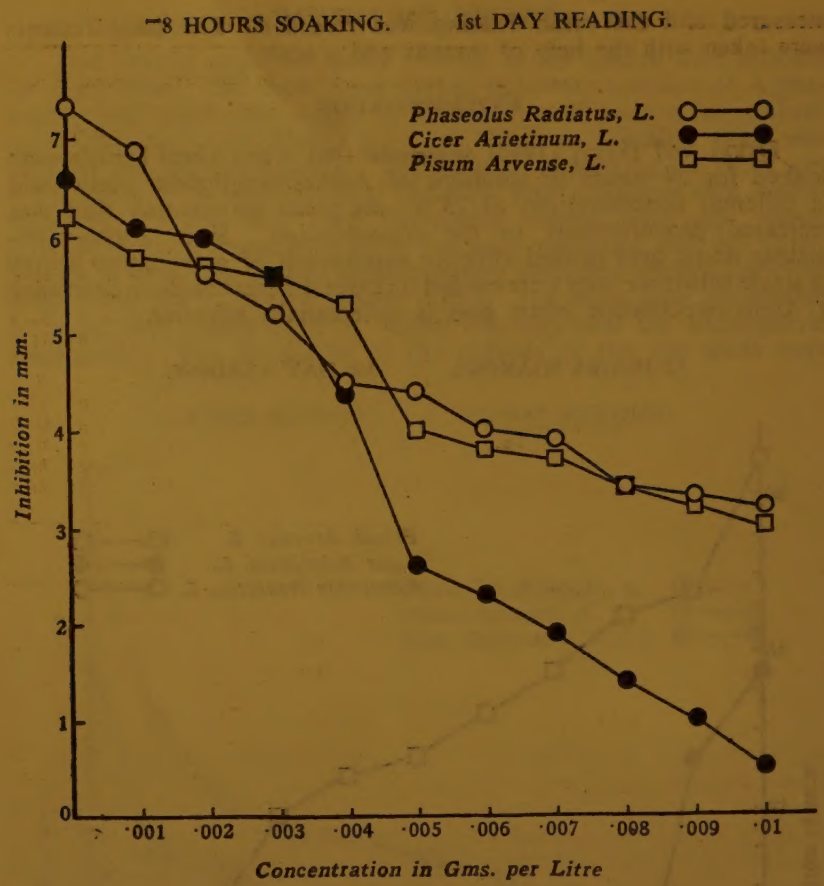


TABLE I. Cicer arietinum *L.*

No. of Expt.	P.P.M.	Mean length of radicles measured in mm. after soaking it for								
		6 hours			12 hours			18 hours		
		1st day	2nd day	3rd day	1st day	2nd day	3rd day	1st day	2nd day	3rd day
1	Dist. water	5·9	18·4	28·8	15·5	28·2	50·1	6·6	16·7	27·8
2	1	5·8	16·1	27·8	13·3	27·0	39·7	6·1	12·8	24·8
3	2	5·7	13·8	25·4	6·0	21·7	39·6	6·0	9·1	21·3
4	3	5·7	13·5	25·0	3·7	16·5	29·8	5·6	7·1	20·4
5	4	5·5	13·0	24·4	3·1	10·2	19·1	4·4	4·7	13·5
6	5	5·4	12·3	23·8	2·7	8·6	14·3	2·6	3·0	9·9
7	6	2·8	12·0	23·4	2·6	8·5	12·6	2·3	2·7	9·0
8	7	2·6	10·5	23·4	2·6	8·2	11·7	1·9	2·5	7·7
9	8	2·5	8·9	22·1	2·1	7·6	10·1	1·4	1·7	7·0
10	9	1·9	8·0	20·8	1·7	5·9	8·9	1·0	1·3	5·4
11	10	1·1	6·5	15·0	1·5	5·4	7·2	0·5	1·0	4·7

TABLE II. Pisum arvense *L.*

No. of Expt.	P.P.M.	Mean length of radicles measured in mm. after soaking it for								
		6 hours			12 hours			18 hours		
		1st day	2nd day	3rd day	1st day	2nd day	3rd day	1st day	2nd day	3rd day
1	Dist. water	16·0	24·0	37·2	20·9	29·9	47·4	6·2	28·3	40·8
2	1	10·7	23·4	36·3	17·6	25·9	33·0	5·8	38·3	39·6
3	2	10·2	23·0	31·6	17·0	25·3	32·9	5·7	28·2	39·4
4	3	9·5	22·4	29·2	15·6	24·5	31·7	5·6	22·6	34·2
5	4	8·9	22·2	29·0	14·6	22·9	31·3	5·3	17·8	28·2
6	5	7·9	22·0	28·6	13·5	22·7	28·3	4·0	17·8	28·0
7	6	7·4	20·8	27·6	13·0	22·5	27·2	3·8	17·0	25·5
8	7	7·2	20·4	27·2	12·0	22·2	25·7	3·7	16·6	25·2
9	8	4·3	19·9	24·7	9·6	21·9	24·4	3·4	14·8	25·0
10	9	4·1	19·6	24·4	8·8	19·7	24·2	3·2	14·3	24·2
11	10	3·6	19·4	23·2	7·1	19·7	23·2	3·0	11·7	24·0

TABLE III. Phaseolus radiatus, *L.*

No. of Expt.	P.P.M.	Mean length of radicles measured in mm. after soaking it for								
		6 hours			12 hours			18 hours		
		1st day	2nd day	3rd day	1st day	2nd day	3rd day	1st day	2nd day	3rd day
1	Dist. water	19·0	24·4	41·0	8·8	24·9	46·3	7·3	29·4	44·4
2	1	14·1	23·4	37·6	4·7	17·5	31·1	6·9	26·2	43·8
3	2	11·9	22·6	37·6	4·5	17·0	34·8	5·6	23·8	36·1
4	3	11·2	20·3	34·9	4·2	15·5	34·6	5·2	20·1	32·8
5	4	11·1	19·9	34·4	3·9	15·3	34·2	4·5	18·6	30·4
6	5	10·5	19·6	33·0	3·8	14·5	26·6	4·4	18·6	27·8
7	6	10·0	19·4	32·4	3·7	13·0	26·5	4·0	18·4	26·8
8	7	9·5	19·0	32·4	3·6	12·9	24·5	3·9	16·6	23·4
9	8	7·8	18·8	31·4	3·5	12·7	24·4	3·4	15·0	23·4
10	9	7·2	17·6	30·6	3·4	12·6	24·4	3·3	14·6	23·1
11	10	7·1	15·6	29·0	3·1	12·5	24·3	3·2	12·5	22·2

The experimental data (Tables I, II and III) are given below and graphs drawn to it are attached herewith.

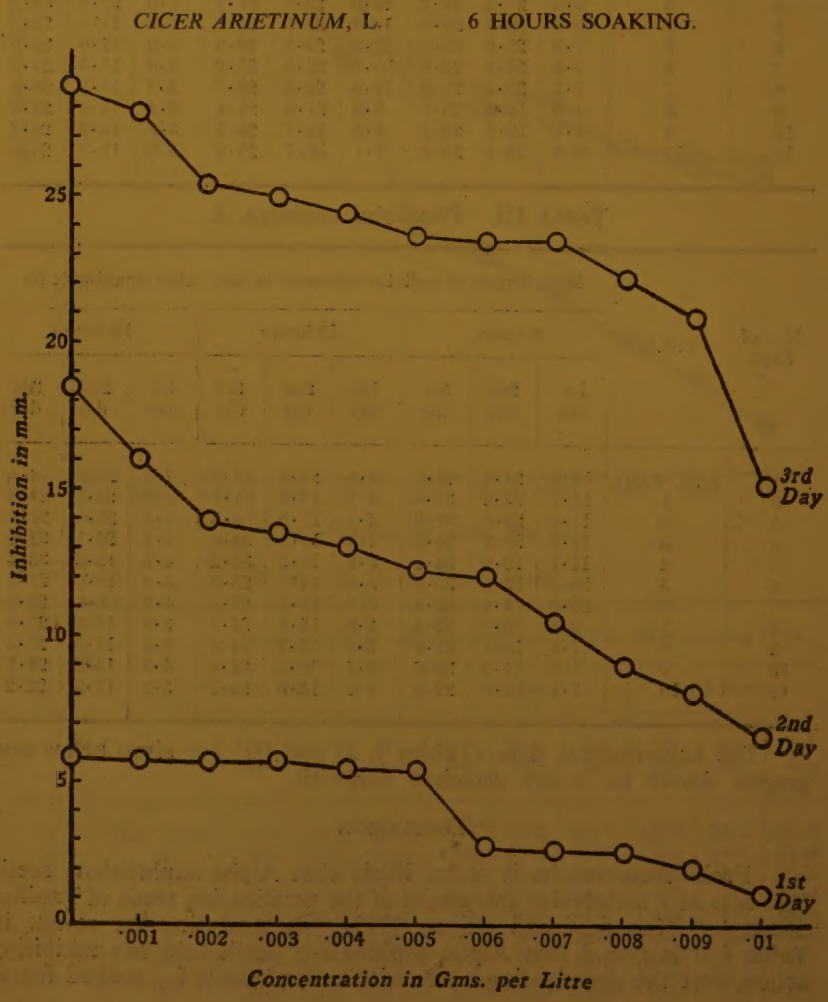
DISCUSSION

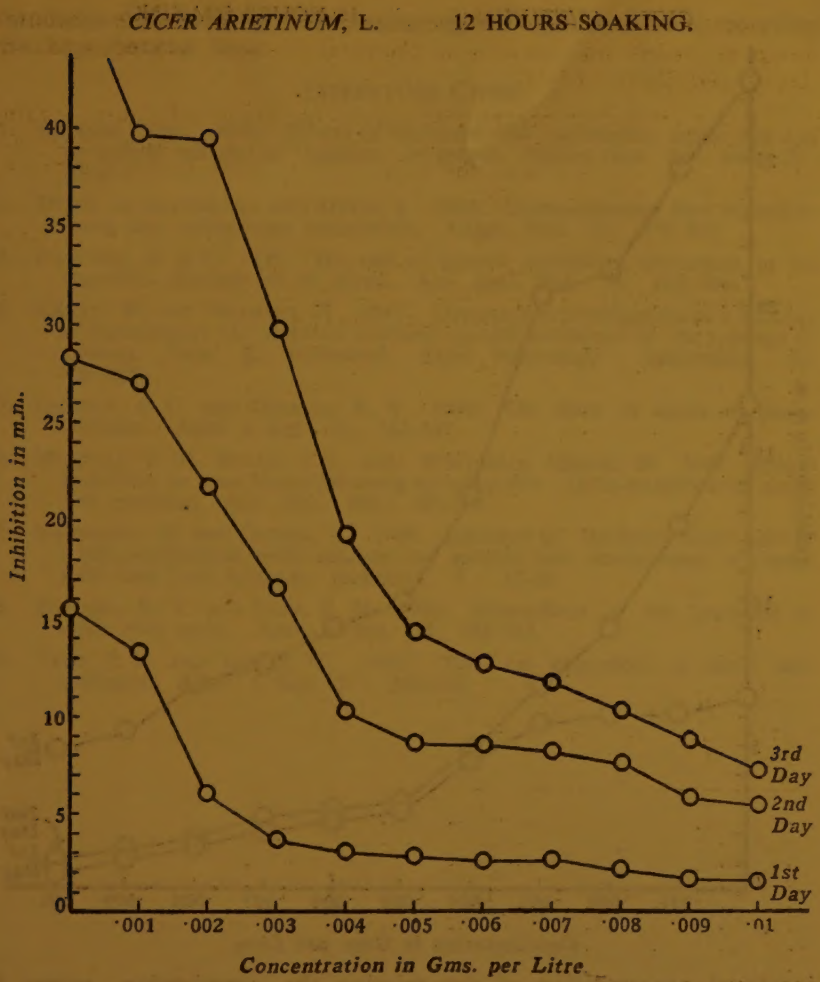
From these results it seems likely that Alpha-naphthalene acetic acid acts as a metabolite antagonist of the germinating seeds of Papilionaceæ (*cf.* Naundorf and Oliver, 1949). By analysing the results in Table I it is found that Alpha-naphthalene acetic acid has inhibiting action over the germinating seeds of *Cicer arietinum* L., soaked for 6,

12 and 18 hours. But as the concentration is raised the inhibition becomes gradual and uniform in the three types of soaked seeds (*cf.* Dykyj and Dykyj, 1943).

Optimum growth on the first day is obtained in 12 hours' soaking in case of *Cicer* and *Pisum* and on 6 hours' soaking in case of *Phaseolus* both in distilled water and the solutions. Growth is retarded by all the solutions in all the cases.

Hence, inference may be made that the more the seed is soaked in growth promoting hormones the more is the diffusion and more is the effective range of inhibition. It appears from the reading of the control experiment that prolonged soaking has deleterious effect on

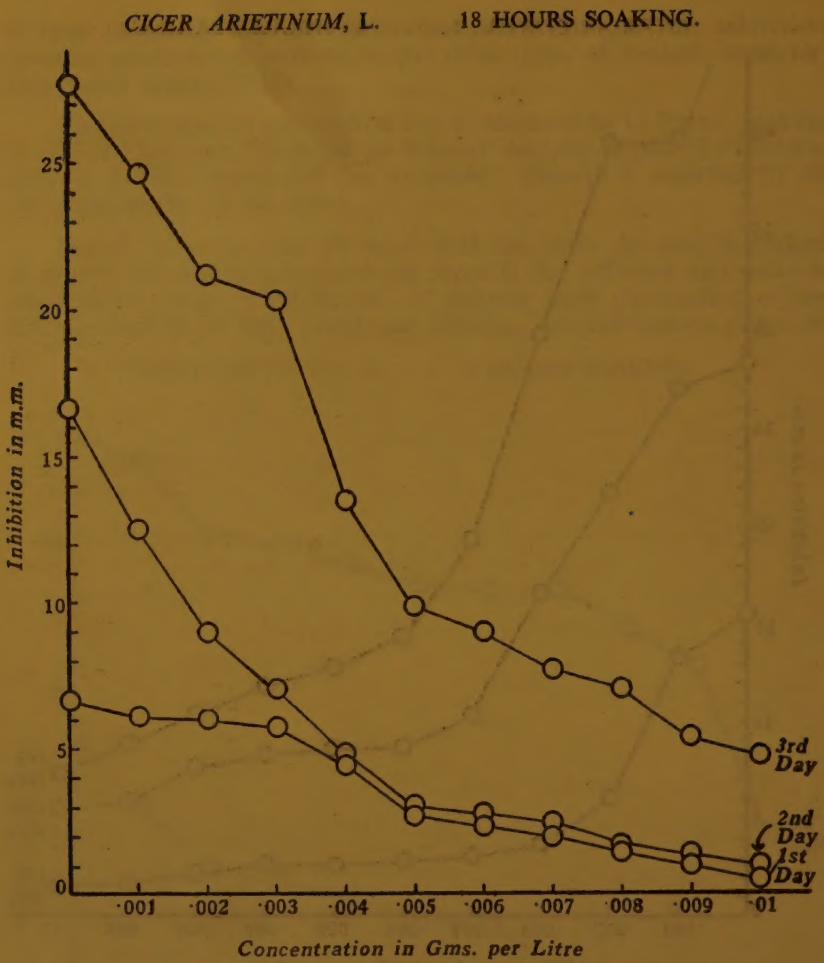




the rate of growth. It is just possible that anaerobic respiration is taking place in prolonged soaking and the general metabolism is affected. So the inhibiting effect of hormones does not manifest itself.

Conclusion and Summary

Growth promoting Alpha-naphthalene acetic acid (Hatcher, 1949) has retarding effect on the metabolism of the germinating seeds. It has gradual inhibiting effect on germination. The rate of inhibition is effected by the concentration of the hormone solutions. As doses are increased the inhibitory effect becomes more and more pronounced leading to the eventual suppression of normal growth. In all trials the mean germination rate is decreased. Hence the mean germination time is increased as an effect of growth hormone. It follows that with



increased concentration of the solution the germination energy is decreased. So it may be concluded that the decrease of the germination energy corresponds directly to the permeability of the growth substance into the kernel. It is noticeable, however, that if the period of soaking is increased from 6 hours to 12 hours inhibitory effect goes on increasing but the effect becomes less pronounced if soaking period is 18 hours. This is possibly due to anaerobic respiration on long soaking, affecting general metabolism, thereby counteracting the effect of the hormone.

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RECENT ADVANCES IN THE STUDY OF SOIL-BORNE FUSARIA

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Notwithstanding the many recent reviews on soil-borne diseases of crop plants, not much attention has been paid to the role played by the genus *Fusarium* in many parts of the world. Much of the earlier work was confined to symptomatological and etiological study of many species of this genus on their hosts of choice. In recent years, however, with the stimulus given by the work of Gäumann and Brian (Gäumann, 1950, 1951 *a*, 1951 *b*; Brian, 1949, 1951, 1952), the whole outlook has to be re-orientated so as to give such studies on soil-borne diseases a physiological bias, and investigate the important question of toxin production by Fusaria and their systemic invasion in vascular plants. The rhizosphere microfloras of soils harbouring these pathogens as either root inhabitants or soil inhabitants and their inter-relationships with the other micro-organisms of the soil also needs study. Concurrent with this new approach, it would be essential to concentrate on the impaired host physiology as a consequence of the disease syndrome resulting from vascular invasion by the pathogen or its metabolites. To this overall picture will have to be added the growth requirements of this genus which it seems to derive from its host in the rhizosphere region, although many species hitherto tested have indicated autotrophy for growth factors (Lilly and Barnett, 1951). The purpose of this review is mainly to summarise the work done on tropical cultivated soils in this laboratory in relation to soil-borne Fusarioses with a view to stimulate further work on the fundamental question of host-parasite relationship and also the behaviour of Fusaria in soils.

Status of Fusaria in Southern Indian Soils

Thirteen distinct species and six varieties of Fusaria have been isolated from Indian soils (Subramanian, 1951 *a*, 1952 *a*, 1952 *b*; Zachariah, 1949; Venkat Ram, 1954) collected from different regions in Southern India. That these Fusaria occur regularly in Southern Indian soils cannot be disputed (Subramanian, 1952 *a*, 1952 *b*; Zachariah, 1949; Sarojini, 1950; Muthukrishnan, 1952; Ramakrishnan, 1953; Agnihotrudu, 1954; Venkat Ram, 1954). Nevertheless, their isolation from such soils has been difficult (Subramanian, 1951 *a*; Zachariah, 1949; Ramakrishnan, 1953). The root burial technique (Sadasivan, 1939; Walker, 1941; Subramanian, 1946, 1950; Zachariah, 1949), however, has been largely the mainstay in evaluating the presence of these pathogens in such soils although it must be stated that this technique leaves much to be desired since it does not give a quantitative picture of the distribution of spores, mycelia, etc., in various

regions in the soil profile with or without crop plants (Stover, 1953 *a*). Of primary importance in the use of this technique, as already indicated by Walker (1941) and by Zachariah (1953), is the judicious selection of surface-sterilising agents with least fungistatic but efficient bacteriostatic properties. It may be of interest here to mention that there is need for further investigation on the use of chemotherapeutic and antibiotic substances with negligible fungistatic properties, the judicious use of which would improve this technique further. Another point of importance which emerges from this study relates to the selective colonisation of dead plant debris in soils by different Fusaria. For instance, *Fusarium udum*, the causal agent of red gram (*Cajanus cajan*) wilt, does not colonise plant debris in the soil under different soil conditions so far tested, and can survive only on tissues already invaded as a pathogen (Agnihotrudu, 1954) and, indeed, freely produces an abundance of sporodochia in such naturally diseased material (Subramanian, 1954). On the other hand, *F. vasinfectum*, the causal agent of cotton wilt, *F. moniliforme*, causing foot rot of paddy, and several Fusaria (Subramanian, 1952 *a*; Shanta, 1953; Stover, 1953 *b*; Venkat Ram, 1954) can colonise plant debris. It is, therefore, obvious that Fusaria belonging to the *F. udum* class could be assigned to the ecological group of root inhabitants as defined by Garrett (1950, 1951). The majority of Fusaria so far studied in this laboratory appear to fall into the soil inhabitant class, and behaviour as typified by *F. udum* appears to be comparatively rare in this genus, although the discovery of more species falling into this group cannot be ruled out and will doubtless be the centre of interest to soil micro-ecologists working on tropical and temperate soils since it signifies specialisation of a somewhat uncommon type for facultative parasites.

At this stage it will be necessary to mention another aspect to this ecological complex, *viz.*, the inability of certain Fusaria, studied here, to make free spread in unsterilised soils. Despite the differences between *F. udum* and *F. vasinfectum* mentioned above, they nevertheless present a comparable behaviour in so far as it concerns their inability to make free spread in unsterilised soils (Subramanian, 1950; Thankam, 1949). To what extent this behaviour could be attributed to the presence of an active competing microflora or their metabolites needs further investigation. Evidence so far obtained in the former field indicates on a statistical basis that any change brought about in microbial numbers either by addition of an active micro-population from pure cultures or by increasing soil fertility by trace element amendments (Thankam, 1949; Sadasivan, 1950, 1951; Sulochana, 1952 *a*, 1952 *b*) brings down the survival and saprophytic activity of Fusaria in soils.

A third aspect to this problem has been the interesting finding of Venkat Ram (1952) that *Fusarium solani* isolated from soil showed increased production of chlamydospores in pure culture and in soil in the presence of either living cultures or filtrates of *Bacterium lichenoides*. This observation leads one to conjecture whether the presence of *B. lichenoides* or its metabolites acts as either a growth promoter increasing chlamydospore formation or as an inhibitor of mycelial growth. Whatever be the interpretation, it is clear that the longevity

of *F. solani* and possibly other Fusaria also in soils through the resting thick-walled chlamydospores seems to be at this stage more than a probability and certainly seems to depend on the associative microfloras with which they occur.

This consideration of *Fusarium* persistence in soils by chlamydospores logically leads one on to the Canadian work on the 'wild type' in the genus *Fusarium* (Miller, 1945; 1946 *a*, 1946 *b*). The results presented by Miller at first sight appeared broad-based notwithstanding the fact that the work of Snyder *et al.* (1949) indicated that the Fusaria associated with mimosa wilt, sumac wilt and pine pitch canker occur as a number of distinct natural clones and that there is a multiplicity of morphologic clones of certain Fusaria in nature. Further work in this laboratory (Subramanian, 1951 *b*, 1954) on many Fusaria isolated from different crop plants and also from soils showed that the majority of the isolates did not fall in line with Miller's 'wild type' concept, largely because a very low percentage of isolates were sparsely sporulating mycelial forms, all the others being in a state of "hokchultur". This being so, the generalisations of Miller and particularly his suggestion to revise *Fusarium* nomenclature was recorded as untenable at this stage. It must, however, be stated that *Fusarium* taxonomists have to and must depend on fresh isolates or 'wild types' of the fungus—a necessary precaution which is the outcome of Miller's thought-provoking study on muskmelon Fusaria—for classification and specific determination of this difficult genus. Apart from the importance of these findings to taxonomy, it may be mentioned here that these observations together with the earlier results summarised above on the formation of sporodochia by *F. udum* in red gram stubble naturally infected during its pathogenic phase and the finding that chlamydospore formation in *F. solani* increases in the presence of *Bacterium lichenoides* or its metabolites indicate that the 'wild type' concept presented here has a bearing on the perennation of these pathogenic fungi in soils. Subramanian (1951 *b*) further stated that forms with abundant aerial mycelium and poor or no sporulation like Miller's 'wild type' do occur in nature, nonetheless they do not appear to be the only form, and indeed the typically mycelial form with poor sporulation and the typically sporodochial or pionnotal form with little or no aerial mycelium have been recorded together with a large number of intergrading forms between these two types. This view is now shared by Buxton (1954) and Buxton & Robertson (1953) working on *Fusarium oxysporum* f. *gladioli*. The occurrence of such a variety of forms in nature inevitably points to the possibility of direct microbial antagonism, *in situ* production of toxins, growth substances or growth factors that decide the biologic and ecologic status of these fungi in soils and will, doubtless, be followed with interest by soil microbiologists.

Fusaria as Soil-borne Pathogens

There seems little doubt at present that many Fusaria can perennate in varied soil types and environments through many growing seasons. *Fusarium vasinfectum*, *F. udum* and *F. moniliforme* occurring in India and studied here for many years, indicate that they are no exception

to this rule. They largely occur in either neutral or alkaline soils of heavy texture and seem to tolerate summer soil temperatures as high as 50° C., remaining viable for long periods beyond one year, at different depths in the soil down to 36″ and below (Sadasivan, 1950). Most of them readily colonise dead plant material with the exception of *Fusarium udum* and appear to be good cellulose decomposers (Siu, 1951). Their survival and pathogenicity do not seem to be curtailed by altering physical conditions of the soil like moisture or amending the soils with organic or inorganic major elements except that, in the case of *F. vasinfectum* wilt of cotton, potash and organic matter control wilt. The main reason for *Fusarium* longevity seems to be its efficiency in colonising cellulose substrata and must be presumed to stay there as long as the organic matter sustains the nutritional needs, as otherwise the microflora of almost all normal soils tested by the Cholodny slide technique indicate rapid disappearance of Fusaria in as early a period as 25–30 days under optimum moisture conditions (Thankam, 1949; Subramanian, 1950). This mode of survival in infected and colonised stubbles offers a special advantage to this genus and seems to be an adaptation to overcome competition from the surrounding microflora of the soil. The results obtained in this laboratory during the past decade by many workers and involving microbiological analysis of many sporadic samples of soils collected from various localities, with different crops, from different depths, and successive samples during different seasons of the year, all favour the above interpretation. All this quantum of work emphasises the futility of any one control measure like crop rotation successfully breaking the saprophytic and parasitic phase of soil-borne Fusaria, and more particularly those belonging to the soil inhabitant class like *F. vasinfectum.*

However, when the fungus is colonised on cotton stubbles as against the results obtained using the Cholodny technique, loss in viability was recorded sooner in soils receiving organic than inorganic (major element) amendments. This confirms the findings of McRae and Shaw (1933) on the control of redgram wilt (*Fusarium udum*) by organic amendments to soils. This method of conducting viability tests with buried stubbles in 'wilt-sick' soils has been largely used here in the absence of a better method, its limitations being mainly governed by periodic colonisation of the stubbles by the spores of one or more Fusaria of the inhabitant class and the consequential vitiation of 'absolute' viability tests. Future work should aim at the evolving of a technique by the use of either chemotherapeutic or antibiotic agents that would selectively rid the soil of its *Fusarium* population without prejudice to the other normal soil microflora that form the bulk of the ecological group typical of that particular soil so that the viability of a single *Fusarium* species can be studied under strictly controlled conditions, *i.e.*, in an environment which is free from interference from other species of the same genus.

Soil Conditions and the Occurrence of Wilt

Promising results were obtained by trace element amendments to 'wilt-sick' soils not only from the point of view of survival and

colonisation of Fusaria on stubble but also from the point of view of their pathogenicity on their hosts of choice. Since the pioneering work of Millikan (1938) on the control of foot rot of wheat by zinc amendments to soils, no serious thought had been bestowed on the practical application of this method. The exact mechanism or role played by trace elements in the control of soil-borne Fusarioses (wilts of cotton and red gram, and foot rot of paddy) reported so far (Sadasivan, 1951; Shanta, 1953; Varadarajan, 1953) could be viewed from two angles, *viz.*, (*a*) their effect on the general soil microflora including the pathogen and (*b*) their effect on the host, which are discussed together hereunder, since they are interrelated problems.

Working on red gram wilt, Sarojini (1950) showed that addition of trace elements, B, Zn and Mn, either singly or in combination to 'wilt-sick' soils in controlled pot culture experiments, controlled wilt, increased plant vigour, and decreased survival of *Fusarium udum* in 'wilt-sick' soils. Similar results were obtained by Yogeswari (1950) who reported that trace element treated soil containing an inoculum of *F. moniliforme* with paddy seedlings showed that Zn amendment alone controlled foot rot at all concentrations tried, especially at 50 p.p.m. No further protection was obtained by either additional Zn amendments following the initial one or pre-soaking seeds in $ZnSO_4$ solutions prior to sowing. Further work by Shanta (1953) showed that Zn and Mn at levels lower than 40 p.p.m. controlled foot rot of paddy to some extent; Mo had no effect, but B increased incidence considerably. In the case of severe attacks, none of the trace elements were useful. All trace elements (Mn, Zn, B, Mo) brought about a sudden quantitative change of the microflora of the soil, the fungal numbers decreasing and the bacterial numbers increasing, but this sudden change was not permanent, the flora returning to a state of normality rapidly except in the case of Zn and B where stability was reached slower than others. In the rhizosphere of the paddy plant, however, addition of trace elements enhanced fungal numbers but reduced bacterial numbers, the quantitative change being more stable with Zn and Mn than with B and Mo. The survival of *Fusarium moniliforme* in colonised stubble was reduced considerably by addition of B and Zn at 25 p.p.m. for Zn and at all levels for B. It is thus clear that B is useful in the control of the saprophytic phase but not the pathogenic phase of *F. moniliforme*. Working on similar lines, but with greater emphasis on the changes brought about on the soil microflora and its repercussions on Fusaria, Sulochana (1952 *a*, 1952 *b*) found that the colonisation of Fusaria on stubbles and their survival in micro-element amended soils depended on the nature of the micro-element, the interaction of concentrations of micro-elements and periods of incubation as well as the interaction of all three factors. The elimination of the pathogen (*F. vasinfectum*) was hastened markedly over the control in the case of Al, Li, B, Mn and Zn treatments, the last mentioned element being the most efficient. The same worker further reported the effect of Al, B, Co, Li, Mn, Mo, Ni and Zn on soil bacteria, actinomycetes and fungi and found them to be significantly influenced by the amendments. Mn amendment brought about maxi-

mum increase in bacterial numbers followed by B, Zn, Mo and Li in the decreasing order. Ni, Al and Co, on the other hand, reduced bacterial numbers. Significance test indicated that the factors micro-elements, concentrations and incubation periods were markedly significant and also the interactions micro-elements × concentrations, and micro-elements × incubation periods. Actinomycete numbers increased with Li amendments and, to a lesser extent, with Mn, B and Co. A fivefold and threefold increase in fungal numbers were noticed with Li and Mn, Mo, Co, B and Al respectively. However, no such increase was noticed with Zn and Ni. In light of these results, the same worker (Sulochana, 1952 *c*) experimented on the response of cotton plants to Zn, Mn, B, Li and Mo amendments in relation to wilt. The first two elements were beneficial to growth of cotton plants whereas the other three were phytotoxic. However, Zn alone controlled wilt due to *F. vasinfectum*, whereas Mn aggravated it.

In all the above work 'wilt-sick' soils were used in their natural state and were distinctly alkaline. With increasing evidence that many trace elements bring about profound changes in the soil microfloras and the consequent effects on colonisation, survival and pathogenicity of several Fusaria, greater emphasis had to be laid on pH changes in soils, since pH has now come to be regarded as an important factor in the mobilisation of ions, particularly of heavy metals, for plant growth and general microbial activity in soils (Stiles, 1946). Such an investigation was carried out using 'wilt-sick' black cotton soils (pH 8·3) over a range of pH 4·0–8·3 (Varadarajan, 1953). An increase in bacterial and fungal numbers at all levels was recorded in soils amended with B, Mn and Zn, the most significant increase being in plus B at pH 6 and 8·3 and a significant decrease in the numbers of fungi at pH 5 with all the three elements. Both in the rhizosphere and non-rhizosphere regions, an increase in bacterial numbers was recorded in the entire range in all the trace elements with significantly highest numbers in all the Mn treatments. However, Fe/Mn was superior to individual elements in increasing bacterial numbers in the non-rhizosphere soil. Fungi indicated a similar increase in numbers with all trace elements, B recording the highest. Non-rhizosphere microflora in soils with cotton seedlings showed a greater increase in numbers to Fe/Mn than soils without cotton seedlings. Fe and Mn added individually and in combination to wilt infested soil reduced the survival of Fusaria, the Fe/Mn combination being superior to individual elements. Similarly, cotton wilt by *F. vasinfectum* in infested soil at a pH range of 4·0–8·3 amended with Fe and Mn showed minimum wilt percentage and optimum plant growth at pH 6·0 in Fe/Mn treatments. Highest rate of mortality of cotton seedlings irrespective of trace element amendments was at pH 8·3. In all this work the ratio of Fe/Mn seemed to be of the utmost importance and has been determined to be in the ratio of 2 : 1, *i.e.*, 40 p.p.m. Fe/20 p.p.m. Mn.

In a recent communication, Stover (1953 *a*) used micro-element amendments of B, Zn and Mn to banana soils infected with *F. oxysporum* f. *cubense* and concluded that there was no significant effect on colonisation and survival of the banana pathogen on banana roots.

These results are in contrast to what have been reported above. On close examination, it would appear that there are no data on soil reaction which, if high, may retard mobilisation of the heavy metals tried, and the flooding technique used by Stover appears to be the limiting factor in preventing the development and antagonistic activity of the general microflora which seems to be the main *modus operandi* of trace element amendments in retarding *Fusarium* survival. This being so, it may be worthwhile repeating these studies under strictly controlled pH and moisture conditions of the banana wilt soils before any generalisation can be made.

Host Physiology in Relation to Fusarium Wilts

It is needless to point out that a closer understanding of the host physiology of the wilted plants is essential in understanding the host-parasite interactions and the consequential changes in the external and internal symptomatology of such wilted plants. Our present knowledge of the symptomatology of fungal wilts is limited to a descriptive study of external symptoms under varying soil conditions (Atkinson, 1892; Smith, 1899; Butler, 1910; Wollenweber and Reinking, 1935). However, recent work done here on *Fusarium vasinfectum* wilt of cotton has resulted in many interesting observations which are of considerable importance and may be of general application to many well-known specific and non-specific wilts. The work of Kalyanasundaram (1953) showed that vein-clearing was the first visible symptom of wilting simultating potato virus Y infection on *Nicotiana tabacum* var. white burley, Hyoscyamus 3 disease of tobacco, etc. Increase in the age of the host at the time of infection retarded the vein-clearing progress. In cotton seedlings although the vein-clearing was discernible to the eye in cotyledonary leaves, it was not so easily seen by the naked eye in the first leaves, but photographing with tri-colour red filter on a panchromatic plate brought out the symptoms clearly (Satyanarayana and Kalyanasundaram, 1952).* The occurrence of vein-clearing in different leaves in a plant showed no definite scheme of distribution as in the case of viruses; however, leaves in the same orthostichies developed the symptoms before other leaves. This partial development of vein-clearing is attributable to a localised action of the toxin (this will be discussed at length later) and cannot, therefore,

* More recent work by Subba Rao in this laboratory on symptomatology of *Fusarium vasinfectum* infected toxin-invaded cotton plants indicates that under ultra-violet light (using one Philips *UV* lamp, HPW 125 W having bulk of its radiation at 3655 Å and photographing on panchromatic plate with GR3 filter) both the cotyledonary leaves and the second and third leaves on their undersurfaces along the veins as well as the stems of such seedlings exhibit fluorescence. Nevertheless, this phenomenon is not seen in the case of healthy plants. These results are comparable to the *in vivo* changes brought about by the tomato spotted wilt virus (Best, R. J., *Austral. J. exp. Biol. & Med. Sci.*, **14**: 199) on tobacco veins on the undersurface resulting in typical fluorescence. It has also been shown by Subba Rao that aqueous extracts of *F. vasinfectum* infected cotton plants and the dialysed culture filtrates of *F. vasinfectum* grown in Richard's medium fluoresce under ultra-violet irradiation, whereas extracts from healthy plants and the Richard's medium show no such phenomena.

be strictly compared to the spread of plant viruses which depend on the phlœm for their systemic spread. Histochemical changes in the diseased vein-cleared leaves were mainly elongation and shrivelling of the palisade cells and a reduction in number of chloroplastids. Chlorophyll content of diseased leaves versus the healthy determined spectrophotometrically indicated a lower chlorophyll content in the former, with the same relationships between the *a*-chlorophyll and *b*-chlorophyll contents. However, the ratio of *a*- to *b*-chlorophyll was always constant. In the infected leaves, palisade and mesophyll cells indicated loss of function. During the progress of the disease, starch synthesis was inhibited during light periods, although normal translocation continued during dark periods indicating no serious derangement of phlœm function. This question of photosynthesis is closely linked up with ascorbic acid metabolism and, indeed, Kalyanasundaram (1952) showed that in wilting cotton plants there was an initial increase in ascorbic acid content and with the progress of wilt the ascorbic acid content progressively decreased, although the supposed precursor of ascorbic acid, *viz.*, the reducing sugars continued to increase with the progress of wilt. This indicated that there was a breakdown in the synthesis of ascorbic acid independent of the accumulation of reducing sugars.

In the case of red gram wilt (*Fusarium udum*), Satyanarayana and Kalyanasundaram (1952) showed that wilt symptoms did not culminate in vein-clearing as in cotton, although there was a general and well-marked dechlorophyllation indicating a toxæmic condition. Further work by Kalyanasundaram (1952) showed close agreement in the ascorbic acid and reducing sugar changes with the progress of wilt which was comparable in every way with the results already mentioned on cotton wilt. It is, therefore, clear that the *in vivo* changes in host physiology of cotton and red gram are similar despite the fact that the two pathogens have a great deal of specificity in that each one of them is confined to its host and does not appear to have, as far as is known, collateral hosts. They also seem to be at variance in the details of the external symptoms that they produce in their respective hosts.

Work on non-protein N and protein N (NPN and PN respectively) by Satyanarayana (1953) indicated that in the case of cotton wilt the net result of pathogenesis appeared to be a decrease in the available NPN constituents of the host and is suggestive of the operation of the enzyme systems of *Fusarium vasinfectum* (it has recently been indicated by K. Laksminarayanan that there is a powerful endo-cellular oxidative deaminase produced by *F. vasinfectum in vitro*). Further, with the onset of infection there was increased PN synthesis in the roots with a corresponding decrease in the NPN in the shoots, indicating the pathogen's ability to interfere with the proteolytic enzyme systems of the roots of infected plants. These changes were independent of the nutritional levels of the soils used. However, K and Zn amendments to soils resulted in greater accumulation of total nitrogen, particularly NPN in the diseased plants, simulating those obtaining in healthy ones and appear, therefore, to enable the system to overcome the effects of enzymic degradation of PN constituents of the host by the pathogen.

Fungal Metabolites and Plant Wilts

Ever since the discovery of the causal agent of cotton wilt in 1892 by Atkinson much interest has surrounded the actual mechanism by which this wilt takes place and many hypotheses have been put forward from time to time. With few variations the views fall into two broad categories: the vessel-plugging theory and the toxin theory. The evidence on this point in recent years has largely been in support of the latter. For instance, Rosen (1926) attributed cotton wilting by *Fusarium vasinfectum* to toxic metabolites of the fungus, as laboratory grown filtrates of the fungus had at least two substances toxic to cotton, one of which was a nitrite presumably by reduction of nitrate to nitrite by the fungus. Similar results on toxic filtrates were reported by Kulkarni and Mundkur (1931). Two other Fusaria, *F. tracheiphilum* and *F. lycopersici* (from cowpea and tomato respectively) also produced culture filtrates which simulated *F. vasinfectum* toxicity on cotton and this experimental evidence on non-specificity of fungal wilt toxins obtained many years ago (Rosen, 1926) is in conformity with more recent work by Gäumann (1951 *b*) and Kalyanasundaram (1953). Among the earlier workers in this field, Schaffnit and Lüdtke (1932) have to be specially mentioned as they indicated beyond doubt that the centrifuged extract of ground hyphæ of the fungus, *F. vasinfectum* exerted a strongly toxic action on host plants belonging to unrelated genera and families such as clover, peas, beans, chrysanthemum, cotton, tomato and wheat, thus indicating the non-specific nature of the toxins extracted from the hyphæ themselves. They ascribed this toxicity to an amine or combination of amines present in the fungal hyphæ. It is significant that in more recent work no distinction has been made between the toxins derived from within the fungal hyphæ themselves as against those excreted into the medium and, indeed, the emphasis has been on the latter. The rather loose application of the term toxin in phytopathology has been critically examined by Dimond and Waggoner (1953 *b*) wherein they have emphasised the need for introducing a new term, *vivotoxin* signifying the actual *in vivo* production of the toxin. Viewed in the light of this terminology, much of the work on wilt toxins of fungi have been considered by Dimond and Waggoner as falling short of a strict interpretation of what the new term signifies. This new nomenclature promises to clear up much of the confusion now surrounding the specificity and non-specificity of some of the fungal toxins studied in relation to their phytotoxicity to rooted plants and excised shoots.

Be that as it may, it is logical to assume the production of toxins in soils and their uptake by plants. The work of Brian *et al.* (1951) on the movement of griseofulvin into lettuce plants as indicated by the ingenious bio-assay test which they employed by the use of *Botrytis allii* spores in evaluating the presence of antibiotics in the guttation fluid of plants is one such example where the plants had absorbed it through their root systems as part of their normal metabolic uptake. Kalyanasundaram and Lakshminarayanan (1953) reported recently that neat culture filtrates of *Fusarium vasinfectum* grown in synthetic

Czapek's medium and tested on cut shoots of cotton after dialysis produced typical wilt symptoms. Higher dilutions of this dialysed neat filtrate did not produce any symptoms of toxæmia, but induced rooting instead. However, such filtrates when autoclaved produced no wilt symptoms but only profuse rooting in cut shoots at all concentrations. They concluded that the dialysed neat culture filtrate of the fungus probably contained two or more factors, (i) a thermolabile factor producing wilt symptoms at higher concentrations and (ii) a thermostable factor stimulating rooting in cut shoots even at dilutions, and that it is likely that the latter factor is ineffective in the presence of higher concentrations of the former. Further work by Satyanarayana (1953) brought out the fact that the addition of the dialysed autoclaved culture filtrate to artificially infected soil within three days after sowing controlled wilt in cotton. However, this beneficial effect was not seen when the addition of the culture filtrate to the soil was delayed beyond three days, presumably because of the unhampered progress of the secretion of the toxin by the fungal inoculum *in situ* in the soil.

A logical extension of the work of Kalyanasundaram and Lakshminarayanan (1953) by Venkata Ram (unpublished) indicates that the production of a growth factor component in culture filtrates is not peculiar to *Fusarium vasinfectum* alone but is shared by three other species of the Elegans group so far tried, *viz.*, *F. conglutinans* Wr., *F. lini* Bolley and *F. oxysporum* Schl. var. *nicotianæ* John. Dialysed culture filtrates of these species produced profuse rooting in *Solanum melongena* L. As in the case of the toxin these growth substances also appear to be non-specific to the host species.

Outlook for the Future

The centre of research on soil-borne diseases appears to have shifted with the many new and important discoveries reported above from the purely qualitative or quantitative routine investigations of soil microfloras to one of biophysical and biochemical approach. We make bold to say this because the work of Gäumann and Brian, whilst indicating the purely physical changes that take place in the plasma of the cell in the infected plant with the repercussions on the physical structure of the cell colloids, consequent increased transpiration and the loss of turgor (Gäumann, 1951 *a*) and the biochemical and chemical approach of the production and stability of antibiotics in soils and their absorption by plants and their bio-assay by micro-organisms (Brian, 1949, 1951, 1952; Brian *et al.*, 1951; Jefferys, 1952), have not taken into account certain other aspects of study of soil-borne Fusaria and Fusarioses. This lacuna has been filled by a series of investigations in tropical soils reported here and, since much of the material was lying scattered, we have endevoured to bring them together. The pioneering work of Scheffer and Walker (1953), in this field, although representing what might now be called an extreme view as a protagonist of the mechanical plugging theory, has not been considered in any detail here largely because in a recent communication (Gothoskar *et al.*, 1953) that school of workers are inclined to review

their position by planning experimental work of an enzymological nature (Lakshminarayanan, 1953), thus conceding, in other words, possibilities other than the plugging theory.

It appears necessary to bring in for consideration once again the vivotoxin definition of Dimond and Waggoner (1953 *b*). Whilst conceding that such a rigid definition is possible, it is necessary to state that not many fungi studied hitherto could be classified under this category. This is so because the ability to produce phytotoxins *in vitro* in soils and also in the region of the root (vascular wilt Fusaria) *in vivo* are not clearly definable for the simple reason that the enzyme systems attendant on these microbiological processes are far too complex for assessment by any one bio-assay technique so far evolved. For instance, the cellulose decomposing ability of many of the soil-borne disease-producing fungi has been shown to be one of the important methods by which many of them perennate in soils, and when they discard their saprophytic role only to infect root systems is not clear, although much of the earlier work presupposes a wound parasitic character for most of these fungi. It, therefore, follows that the seat of toxin production may not and need not necessarily be inside the host particularly in view of the very many useful investigations done in recent years indicating an intense rhizosphere microfloral complex in the root region of many plants (Katznelson *et al.*, 1948; Agnihotrudu 1953, 1954) culminating in a state in which one could picture a variety of relationships of host and fungus ranging from symbiosis to parasitism.

The role of heavy metals in correcting wilts, reported here, is a departure from recognised methods of study of soil-borne pathogenic fungi, but judging from the somewhat impressive statistical quantitative survey of the behaviour of the general microflora with which these Fusaria live in association (Sulochana, 1952 *a*, 1952 *b*; Varadarajan, 1953), there seems to be a new opening in understanding the inter-relationships of the many associative micro-organisms both in the rhizosphere and the non-rhizosphere soils that contribute to plant well-being. Of equal importance seems to be the need for critically studying the many species of *Fusarium* recorded in many parts of the world from the point of view of the production of growth substances. With this is linked up the question of the utilisation of many heavy metals by host and parasite, as the derangement of the chlorophyll mechanism (as indicated by the ascrobic acid studies reported here) may well be the aftermath of immobilisation of the uptake of these and, in such a state, the enzyme systems and their function on which chlorophyll activity depends in normal plants will inevitably get deranged. Although we have comparatively few results from investigations conducted here on the cause of this derangement but more evidence on its effect on the host plant, further critical work is indicated in this field. In this connection, we have read with interest the work of Dimond and Waggoner (1953 *a*) on lycomarasmin and their estimates of its production *in vivo* in the tomato plant. Their tentative conclusions that they are unable to say that sufficient lycomarasmin is produced *in vivo* to produce *Fusarium* wilt symptoms and also their conclusions

that lycomarasmin under certain conditions decomposes losing both Fe-chelating ability and wilt-inducing ability and that it chelates other metals not toxic to plants further suggesting that lycomarasmin is active on plant cells as the Fe-lycomarasmin chelate complex fit in with some of our own observations on the recovery of cotton plants in 'wilt-sick' soils on the addition of Fe, Mn, K and Zn. These lead us to believe that the results of Dimond and Waggoner are comparable to the wilt-reducing effect of Fe/Mn, K and Zn in the case of cotton wilt and other wilts. The work of Varadarajan (1953) on Fe/Mn in the ratio of 2 : 1 added to 'wilt-sick' soils at pH 6·0 and planted with cotton seedlings showed progressive and typical, wilt symptoms up to the twelfth day of sowing largely confined to vein-clearing and general dechlorophyllation of the first leaves; but such plants made a remarkable recovery resulting in plants indistinguishable from the controls from the 22nd day after sowing. These results raise many fundamental issues such as the role of these heavy metals in antidoting toxins, production of growth factors by Fusaria in soils or *in vivo* in the plant, their role in the nitrogen economy of the plant in roots and shoots and also their effects on fungal enzyme systems.

As a result of extensive chromatographic studies made in this laboratory on the distribution of α-amino constituents in roots and shoots of resistant and susceptible varieties of cotton, Lakshminarayanan (unpublished) reports the interesting fact that cystine is consistently present besides the other constituents in the resistant variety, whereas it is absent in the susceptible variety. He suggests the possibility of the cystine in the resistant variety chelating with Fe^{+++} in the host and rendering it unavailable for forming the complex with the toxin which appears to be a pre-requisite for toxigenic wilting.

In conclusion, it could be said that the study of wilt organisms and their metabolites in their hosts has revealed many new experimental facts of fundamental importance. Future work has undoubtedly to be one of intensive study of the physiological relationships of the pathogen and the host *in vivo*.

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SOME SLIME-MOULDS FROM SOUTHERN INDIA—I

By V. Agnihothrudu

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(Received for publication on April 30, 1954)

In this paper is presented an account of some myxomycetes collected from Madras during the rainy months (September-December, 1953). *Physarum nutans* Persoon and *P. crateriforme* Petch are reported for the first time from India and *P. vernum* Somm. for the first time from Madras State.

1. *Physarum nutans* Persoon in *Usteri. Ann. Bot.*, 1795, **15**, p. 6; Macbride, T. H., *The North American Slime-Moulds*, New ed., 1922, pp. 97–98; Lister, A., *Monograph of The Mycetozoa*, 3rd ed., 1925, pp. 46–48.

Plasmodium not observed. Sporangia gregarious, stipitate, more or less flattened or concave beneath. Total height 750–1,750 μ, diameter 460–750 μ, greyish-white with a delicate peridium impregnated with minute white lime granules. The sporangial wall dehiscing laciniately and exposing the spore mass. Stipe long, tapering towards the sporangial end, upto 1 mm. long, pale brown when young, turning deep brown or fuscous with age, longitudinally striate, containing refuse matter. Lime absent in the stalk. Capillitium consisting of hyaline slender threads branching at acute angles and anastomosing with flat, irregular, rounded or elongated calcareous nodules. Spores clear, brownish-violet, globose to spherical, minutely verrucose, average diameter 9·8 μ; range 8·8–10·8 μ, mostly 10 μ each enclosing a guttule.

Only two collections are preserved, one on decaying leaves of pigeon pea [*Cajanus cajan* (Linn.) Millsp., Agri-Horticultural Society Gardens, Madras, 3–9–1953, coll. V. Agnihothrudu (Herb. M.U.B.L. No. 1081); on decaying cotton bolls, Agri-Horticultural Society Gardens, Madras, 6–9–1953, coll. V. Agnihothrudu (Herb. M.U.B.L. No. 1082).

2. *Physarum vernum* Sommerfelt in litt. *Fr. Syst. Myc.*, **3**, 1829, p. 146; Macbride, T. H., *The North American Slime-Moulds*, New ed., 1922, p. 51; Lister, A., *Monograph of The Mycetozoa*, 3rd ed., 1925, p. 54; Lodhi, S. A., *Publ. of The Univ. of the Punjab*, 1934, p. 5.

Plasmodium white to pale yellow in colour, found on heaps of decomposing plant débris and on the abaxial leaf surface of many weeds growing in the pigeon-pea field in the Agri-Horticultural Society Gardens, Madras. Sporangia gregarious, sessile, globose to subglobose, often confluent forming plasmodiocarps which are short, elongate, straight or sinuous and frequently forming irregular reticulations, white or greyish-white in colour, 480–1,250 μ in diameter, often

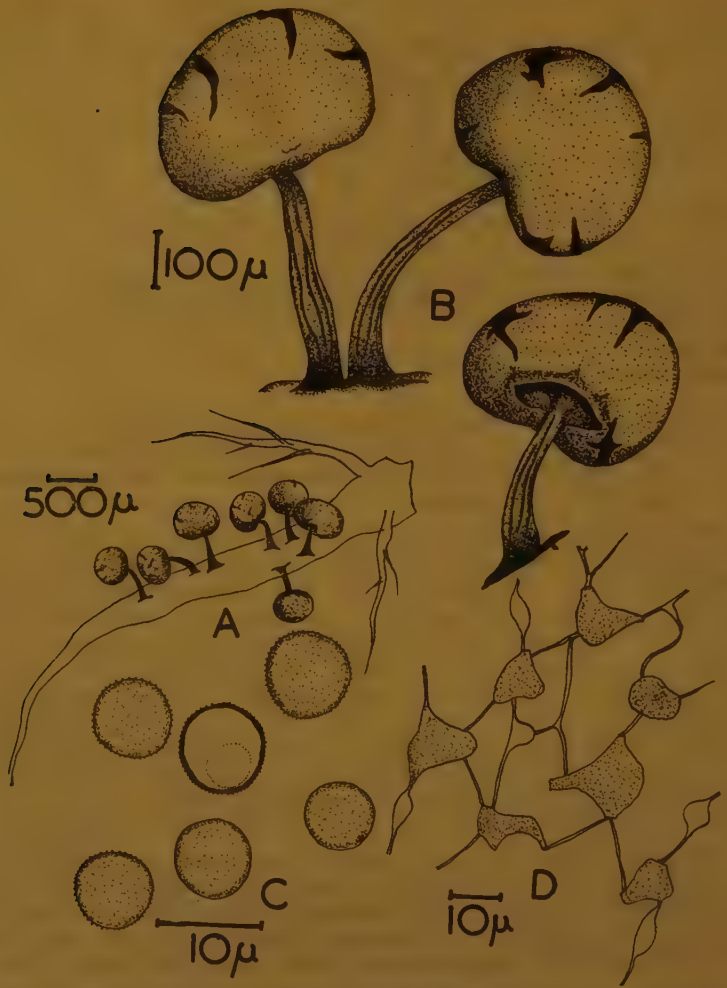

FIG. 1. *Physarum nutans* Persoon. A. Sporangia on the root surface of pigeon-pea plant. B. Two sporangia showing irregular dehiscence of the peridium. C. Spores. D. Capillitial threads and calcareous nodules.

upto 4–5 mm. in length, surface rough or rugulose. Peridium more or less testaceous, breaking irregularly exposing the spore mass. Capillitium consisting of thin hyaline threads highly ramified and anastomosing with angular lime knots. Spores pale purple to purplish-brown in colour, globose to spherical; verrucose; average diameter 11·7 μ, range 8·8–12·4 μ, mostly 12 μ, with pale germinal areas.

On decaying grasses, Madras University Botany Field Laboratory campus, Agri-Horticultural Society Gardens, Madras, 6–10–1953, coll. V. Agnihothrudu (Herb. M.U.B.L. No. 1083); on decaying leaf

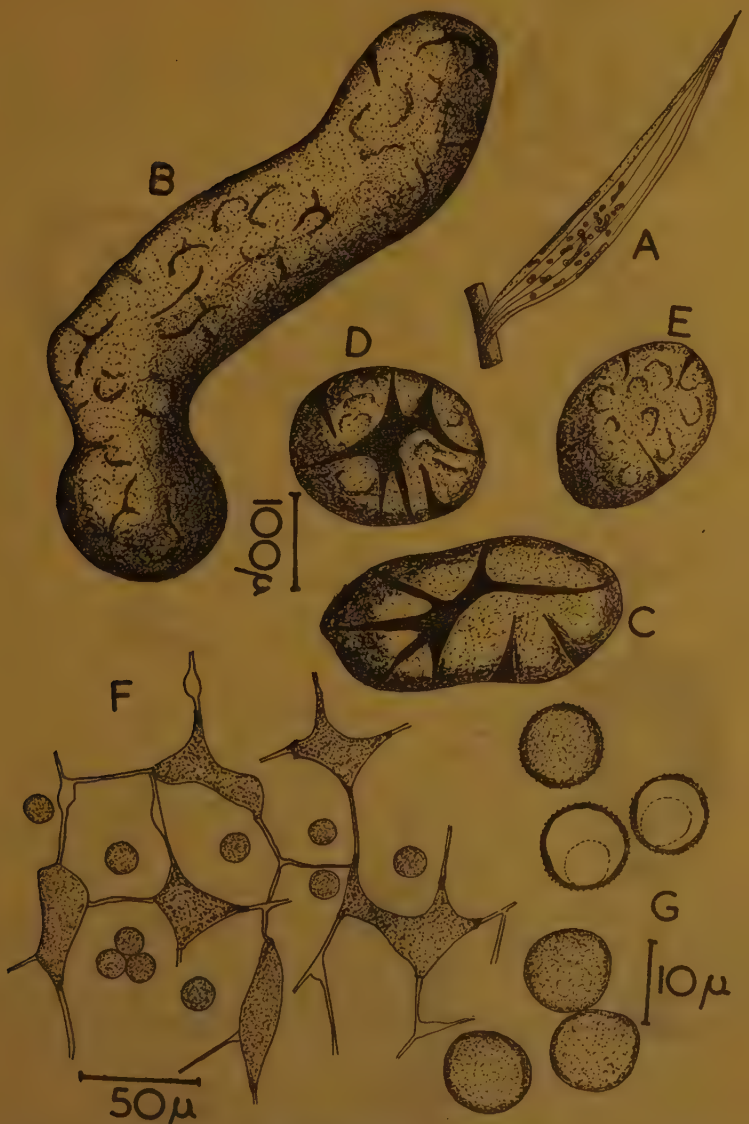

FIG. 2. *Physarum vernum* Sommerfelt. A. Sporangia on the leaf of *Eleusine ægyptiaca* Desf. B & C. Plasmodiocarps. D & E. Individual sporangia showing irregular dehiscence of the peridium. F. Capillitial threads and calcareous nodules. G. Spores.

bases of *Cycas circinalis* Linn., Agri-Horticultural Society Gardens, 6–10–1953, coll. V. Agnihothrudu, (Herb. M.U.B.L. No. 1084); on incubated roots of pigeon-pea [*Cajanus cajan* (Linn.) Millsp.] in the

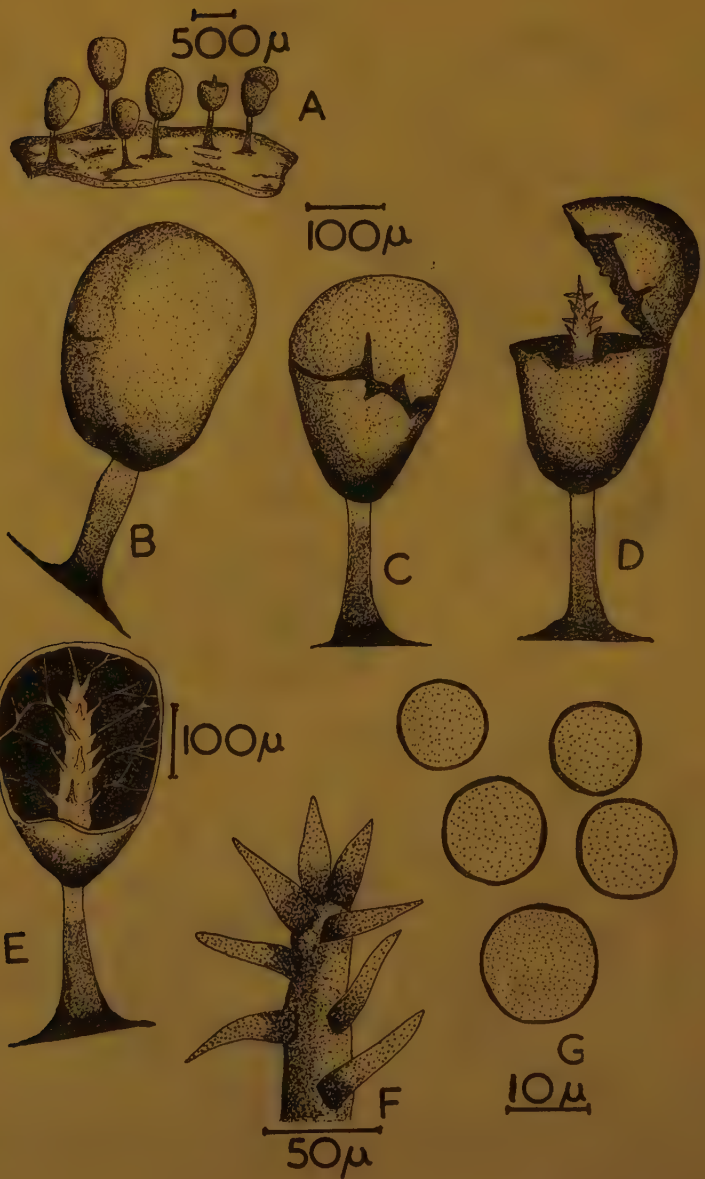

FIG. 3. *Physarum crateriforme* Petch. A. A group of sporangia on the bark. B & C. Two individual sporangia. D. A sporangium showing the irregular circumscisile dehiscence and the columella. E. A sporangium showing the columella with spine-like calcareorus nodules and the capillitial threads. F. The columella with calcareous spines. G. Spores.

Madras University Botany Field Laboratory campus, 14–10–1953, coll. V. Agnihothrudu (Herb. M.U.B.L. No. 1085); on *Eleusine ægyptiaca* Desf. Ayanavaram, Madras, 14–10–1953, coll. V. Agnihothrudu (Herb. M.U.B.L. No. 1086); on stems of *Corchorus trilocularis* Linn., Agri-Horticultural Society Gardens, Madras, 22–10–1953, coll. V. Agnihothrudu (Herb. M.U.B.L. No. 1087); on leaves of *Waltheria indica* Linn., Ayanavaram, Madras, 22–10–1953, coll. V. Agnihothrudu (Herb. M.U.B.L. No. 1088).

3. *Physarum crateriforme* Petch in *Ann. Royal bot. Gardens, Peradeniya*, **4**, 1909, p. 304; Macbride, T. H., *The North American Slime-Moulds*, New ed., 1922, p. 100 (given as an extra-limital species); Lister, *A Monograph of The Mycetozoa*, 3rd ed., 1925, p. 49.

Plasmodium not observed. Sporangia gregarious or scattered white or slightly tinged grey, oval, obovoid, cylindrical or sub-globose stalked. No plasmodiocarps were observed. Sporangial heads measuring 500–780 × 200–400 μ in diameter. Sporangial wall heavily charged with lime granules, dehiscence of the peridium irregularly circumscissile. Stalk opaque, short, stout, conical, black or dark brown below, paler at the sporangial end. Stipe measuring 260–600 μ long and up to 200 μ broad at the base and 80μ at the apex, containing refuse matter. Columella present, variable in shape, white or pale yellow-brown in colour, usually cylindrical, reaching more or less the vertex of the sporangium. The lime knots forming a dense massive columella giving off horizontal spine-like or fusiform projections from the apices of which are given long, hyaline, thin, ramified capillitial threads that are connected to the sporangial membrane. Spores pale purple or dull lilac in colour, spinulose, average diameter 12 μ, range 9·6–12·8 μ mostly 12·7 μ.

Only one collection is preserved. On the bark of *Wrightia tinctoria* R. Br., Agri-Horticultural Society Gardens, 22–10–1953, coll. V. Agnihothrudu (Herb. M.U.B.L. No. 1089).

Acknowledgments

I am indebted to Professor T. S. Sadasivan and Dr. C. V. Subramanian for their helpful suggestions in the preparation of this paper. I thank Dr. K. Ramakrishnan for critically reading the manuscript and the Government of India for the award of a senior scholarship during the tenure of which this work was done.

SOME SLIME-MOULDS FROM SOUTHERN INDIA—II

By V. Agnihothrudu

University Botany Laboratory, Madras-5

(Received for publication on June 10, 1954)

Four myxomycetes, namely, *Fuligo septica* Gmelin, *Physarum nicaraguense* Macbride, *Diderma hemisphericum* (Bull.) Hornem., *Arcyria cinerea* (Bull.) Pers., collected from Madras are described in this paper.

4. *Fuligo septica* Gmelin, *Syst. Nat.*, 1466, 1791; Macbride, T. H., *The North American Slime-Moulds*, New ed., 1922, pp. 27–30; Lister, *Monograph of The Mycetozoa*, 1925, pp. 66–68.

Plasmodium pale yellow, creeping over piles of vegetable débris. Aethalia pulvinate, varying considerably in size, 1·5–3·5 cm. broad, pale yellow-grey in colour. The sporangia that go to form the æthalium are intricately anastomosing, 1·75–2·5 mm. broad. Several air spaces that separate the sporangia pervade the æthalium. Cortex present, moderately thick, pale yellow in colour. Sporangia present a gyrose appearance to the aethalium. Some superficial layers of the fruit body are sterile and contain no spore masses. Sporangial wall membranous, fragile and charged with scattered deposits of lime granules. In older aethalia the superficial sterile sporangia break away exposing the spore masses of the subjacent sporangia. Columella absent. Capillitium scanty, consisting of a loose reticulum of slender hyaline threads, rather expanded at the axils with spindle-shaped calcareous yellow lime knots varying widely in size. These calcareous masses appearing as refractive specks on the aethalium. Spores violet, spherical to subspherical with almost smooth wall measuring average 8·0 μ, range 6·4–8·4 μ, mostly 8·0 μ.

Only one collection is preserved, on decaying vegetable débris in pigeon-pea field, Madras University Botany Field Research Laboratory, Agri-Horticultural Society Gardens, Madras, 10–9–1953, coll. V. Agnihothrudu (Herb. M.U.B.L. No. 1090).

5. *Physarum nicaraguense* Macbride in *Bull. Nat. Hist.*, Iowa, **2**, 1893, p. 382; Macbride, *The North American Slime-Moulds*, New ed., 1922, p. 83; Lister, *Monograph of The Mycetozoa*, 3rd ed., 1925, pp. 52–53; Petch, *Ann. Royal bot. Gardens, Peradeniya*, **7**, 1910, pp. 334–335.

Plasmodium not observed. Sporangia gregarious, multilobate, aggregated in botryose clusters of 4–10 or 12, single isolated sporangia were not observed, obconical, up to 800 μ in diameter, mostly 500 μ with greyish-white thin fragile peridium impregnated with lime deposits,

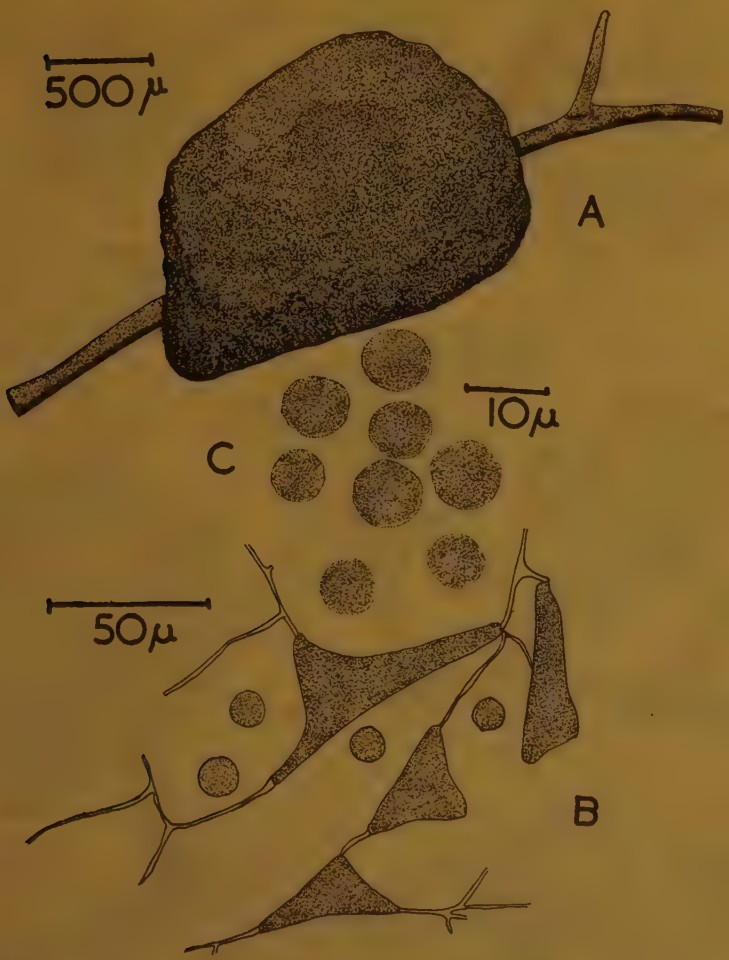

FIG. 1. *Fuligo septica* Gmelin. A. An æthalium attached to a twig. B. Capillitial threads with angular lime knots. C, Spores.

breaking irregularly, walls of the dehisced sporangia show fimbriate margins. Stalks of the sporangia 0·4–1·0 mm. high, twisted, furrowed, brownish at the base, greyish-brown above, enclosing refuse matter in the lower part. The stipe which represents the confluent stalks of the sporangia is a short upward extension or sweep of the common hypothallus which is deep brown or black in colour, prominent in some but insipiently developed in others. Sessile sporangia were not observed. Capillitium consists of thin, hyaline threads attached to angular pale yellow lime knots which were seen to aggregate in the middle of the sporangium to form a rather massive pseudocolumella. Spores purple-brown in colour, spherical, smooth-walled when young

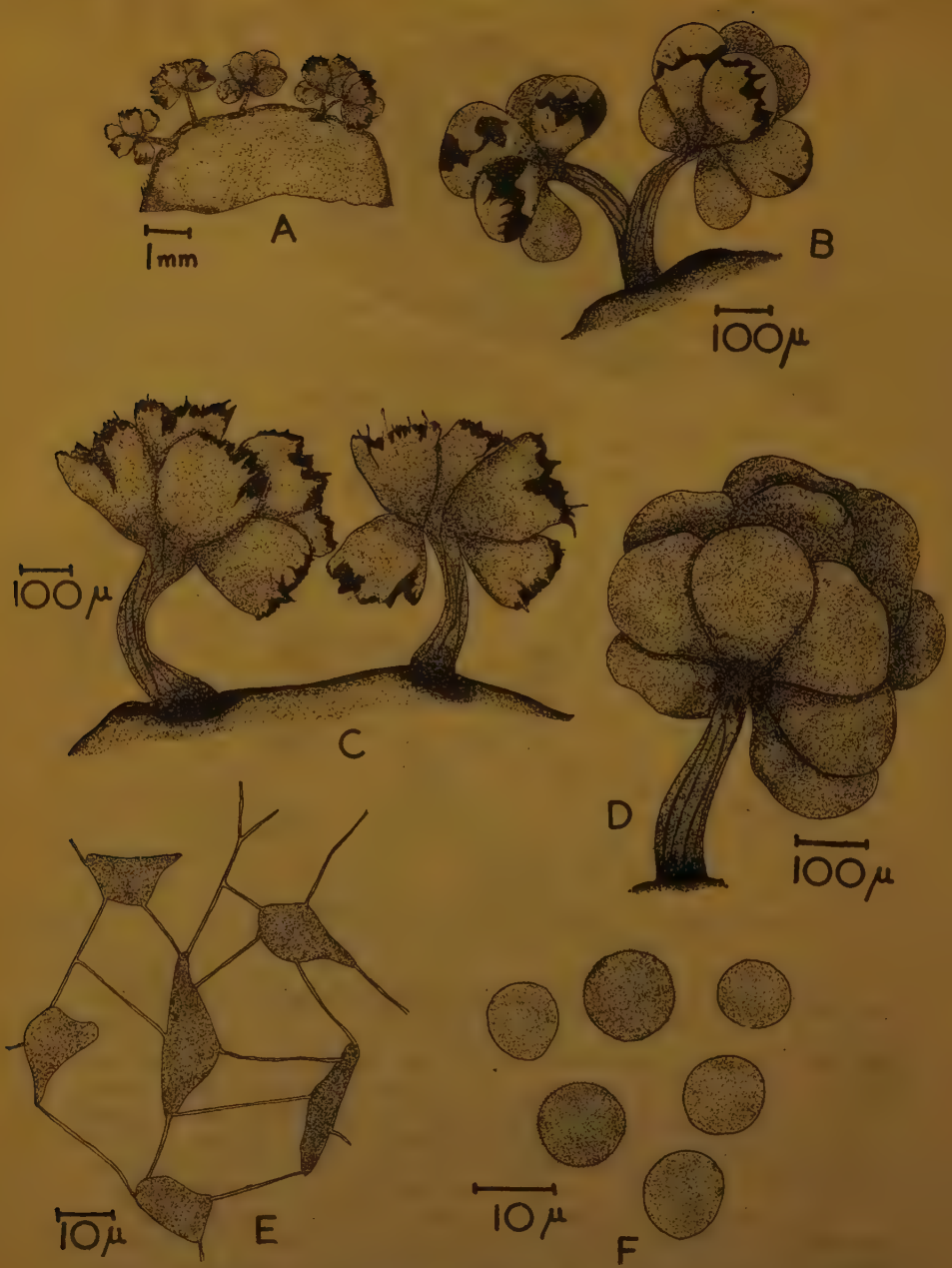

FIG. 2. *Physarum nicaraguense* Macbride. A. A group of sporangia on the leaf base of *Cycas*. B. Sporangia showing the irregular dehiscence of the peridium. C. Completely dehisced sporangia showing the fimbriate margins of the peridial remnants. D. A sporangial cluster with 11 sporangia. E. Capillitial threads with lime knots. F. Spores.

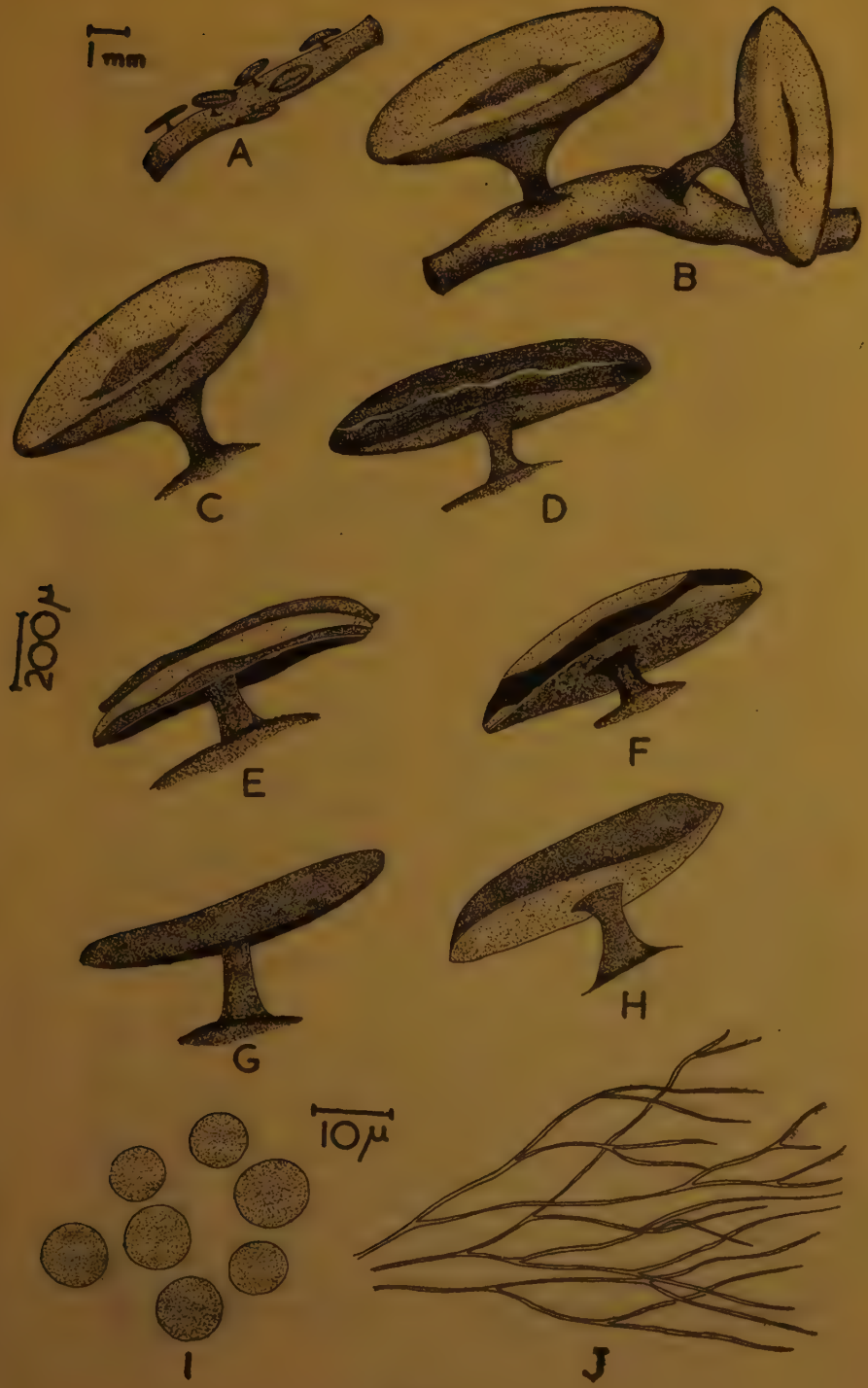

FIG. 3

FIG. 3. *Diderma hemisphericum* (Bull.) Hornem. A. A twig with sporangia. B. & C. Sporangia showing the hypothallus. D. Sporangium showing the marginal dehiscence of the outer peridium. E. Sporangium showing the ruptured outer peridium and evanescent inner peridium. F. Sporangium after complete dehiscence of the inner and outer peridium G. & H. Sporangia with spore mass supported on persistent basal peridium. I. Spores. J. Capillitial threads.

later becoming uniformly echinulate. Average diameter 10·5 μ, range 9·6–12·4 μ, mostly 10·8 μ.

On decaying leaf bases of *Cycas circinalis* L., Agri-Horticultural Society Gardens, Madras, 8–10–1953, coll. V. Agnihothrudu (Herb. M.U.B.L. No. 1091).

6. *Diderma hemisphericum* (Bull.) Hornem., in *Fl. Dan.*, 33, 1829, p. 13; Macbride, *The North American Slime-Moulds*, New ed., 1922, pp. 138–139; Lister, *Monograph of The Mycetozoa*, 3rd ed., 1925, p. 85; Schinz, Myxogastres, *Rabenhorst Kryptogamen Flora*, Abt. X, p. 175.

Plasmodium not observed. Sporangia gregarious, orbicular, disciform, stipitate, slightly umbilicate and depressed above. Stipe measuring up to 1 mm. in length. No sessile sporangia were observed, but in some cases the stalks were very short, measuring only 150 μ in length. Sporangia chalk-white. Sporangial wall composed of two layers, the outer peridium white, fragile, crustaceous, charged with globular calcareous crustations, usually breaking along the margins of the discoid sporangia, and separating from the inner peridium which is delicate, cinereous, dehiscing laciniately exposing the spore mass. Stipe short, ochraceous, thick up to 500 μ in diameter, furrowed in few cases only, densely impregnated with lime granules. Columella not distinct from the hard calcareous base of the sporangium. Capillitium composed of delicate threads up to 2 μ thick, anastomosing, colourless and often very scanty. When mature peridial wall drops off completely exposing the spore mass supported on discoid base of the sporangium. After the spore dispersal the persistent stalks of sporangia are seen surmounted by the disc which represents the consolidated calcareous columella, the expanded top of the stipe and partly persistent basal peridium of the rotund sporangium. Spores dilute violet-brown, smooth, measuring average diameter 8·8 μ, range 6·8–9·6 μ, mostly 9·0 μ.

On leaves of *Eleusine aegyptiaca* Dest. Presidency College campus, Madras, 7–11–1953, coll. V. Agnihothrudu (Herb. M.U.B.L. No. 1092); on decaying twig of *Croton sparciflorus* Morung, Ayanavaram, Madras, 15–11–1953, coll. V. Agnihothrudu (Herb. M.U.B.L. No. 1093); on incubated roots of *Crotalatia juncea* L., Madras University Botany Field Research Laboratory, Agri-Horticultural Society Gardens, Madras, 10–9–1953, coll. V. Agnihothrudu (Herb. M.U.B.L. No. 1094); on decomposing leaves of *Salvia* sp. My Ladye's Garden, Madras, 22–10–1953, coll. V. Agnihothrudu (Herb. M.U.B.L. No. 1095); on the lower surface of the fronds of *Pleopeltis* sp. My Ladye's Garden Madras, 22–10–1953, coll. V. Agnihothrudu (Herb. M.U.B.L. No. 1096); on the lower surface of the leaves of *Alpinia* sp. Agri-Horticultural

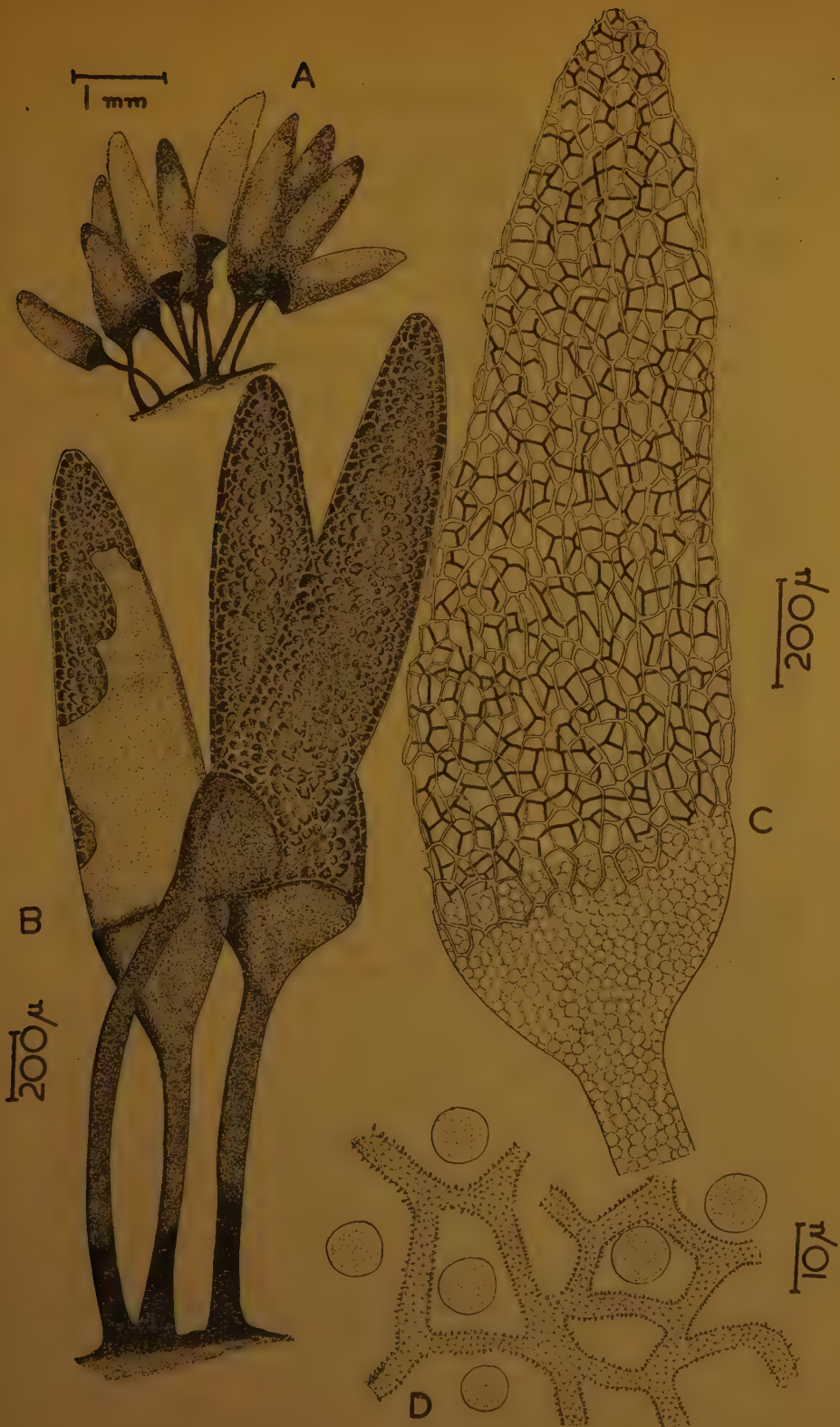

FIG. 4

FIG. 4. *Arcyria cinerea* (Bull.) Persoon. A. A group of sporangia. B. Sporangia showing evanescent peridium, the reticulate capillitium and the hypothallus. C. Calyculus and the capillitial reticulum. D. Capillitium and spores.

Society's Gardens, Madras, 28–11–1953, coll. V. Agnihothrudu (Herb. M.U.B.L. No. 1097).

7. *Arcyria cinerea* (Bull.) Persoon in *Syn. Fung.*, 1801, p. 184; Macbride, *The North American Slime-Moulds*, New ed., 1922, p. 254; Lister, *Monograph of The Mycetozoa*, 3rd ed., 1925, pp. 231–233; Lodhi, S. A., Indian Slime-Moulds, *Publ. of the Univ. of the Punjab*, 1934, p. 23; Bruhe and Sen Gupta, Indian Slime-Fungi (*Myxomycetes* or *Mycetozoa*), *J. Dept. of Sci.*, Calcutta University, **8**, 1927, p. 121.

Plasmodium creeping, greyish-white in colour, sporangia gregarious, ovoid, cylindrical, usually cylindrical at the base tapering upward into a blunt apex, about 1–4 mm. high, stipitate, pale yellowish-grey fading to dull yellow. Peridium extremely thin and evanescent. Cup of the sporangium or the calyculus very small, thin, membranous, smooth, pale yellowish in glycerine jelly mounts, filled with spore-like mass of isodiametric cells. Capillitium consisting of a close reticulum of threads varying in diameter from 2–4 μ, yellowish, minutely spinulose and attached at many places on the inner aspect of the calyculus. Spores almost colourless, smooth-walled, measuring on average 7·4 μ, range 6·4–8·4 μ, mostly 7·6 μ.

On the decaying leaf bases of *Cycas circinalis* L., Agri-Horticultural Society Gardens, Madras, 20–10–1953, coll. V. Agnihothrudu (Herb. M.U.B.L. No. 1098).

ACKNOWLEDGMENTS

I am indebted to Professor T. S. Sadasivan and Dr. C. V. Subramanian for their helpful suggestions in the preparation of this paper. I thank Dr. K. Ramakrishnan for critically reading the manuscript and the Government of India for the award of a senior scholarship during the tenure of which this work was done.

A NEW SPECIES OF *TRICHOCONIS* ON *CROTALARIA VERRUCOSA* L. FROM HYDERABAD-DN.

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Botany Department, Osmania University, Hyderabad-Dn.–7

(Received for publication on June 2, 1954)

Crotalaria verrucosa L. plants showing shrivelling and discolouration of leaves and pods were collected from the vicinity of the Agricultural College Farm, Osmania University. A fungus was isolated from the diseased material. A description of the fungus based on a detailed study of several isolates is given below.

The fungus appears externally as 'white mould' in the form of irregular, whitish, cottony patches on leaves, flowers and mature pods causing shrivelling and discolouration of these parts. In severe attacks the fungus invades the seeds also and causes their discolouration. The mycelium is well-developed, olive brown. The hyphæ are 3·6–6 μ in thickness, septate when mature at intervals of 18–36 μ, and branch at right angles. The conidiophores are not sharply distinguishable from the hyphæ, and are erect and septate with swollen ends. Each conidiophore bears apically one conidium. The conidia are dark brown, elongate, fusiform, 5–8-septate, somewhat thick-walled, and attached by their broader ends. Each conidium is provided with a long, aseptate appendage at its apex. The conidia measure 46·8–95·0 × 10·4–18·2 μ and the appendages 49·4–127·5 × 1–2·6 μ. Numerous sclerotia are produced in pure culture. These are deep black, cylindrical or somewhat irregular, and provided with numerous stiff, hyaline bristles.

The dark brown fusiform, many-celled conidia with apical unbranched appendages, produced singly on poorly differentiated conidiophores led us to place the fungus in the genus *Trichoconis* Clements. Two species of this genus are known: *T. caudata* (App. & Str.) Clements (Clements, 1909), and *T. padwickii* Ganguly (Ganguly, 1947). Our fungus was found to differ from both these species in spore and sclerotial size and in certain other characters. The conidia in our fungus are very much longer than those of *T. caudata* and a little longer than those of *T. padwickii*. They are 5–8 septate here while those of *T. caudata* are 2–4 septate and those of *T. padwickii* are 3–5 septate. The sclerotia are very much larger and different in shape in the present fungus. The appendage in the present fungus is longer than the spore and nonseptate unlike in *T. padwickii* where it is as long as the spore and septate. Finally our fungus infects a totally different host. It is, therefore, considered distinct from *T. caudata* and *T. padwickii* and described as a new species.

FIG. 1.

FIG. 2.

Trichoconis crotalariæ Salam & Rao sp. nov.

Spots irregular, white, mycelium well-developed, hyphæ 3·6–6 μ thick, branched, septate when mature at intervals of 18–36 μ; branches at right angles to the main axis; conidiophores not sharply distinguishable from hyphæ, erect, septate, with swollen apex, 52–156 μ long, 3·6–6 μ broad, bearing one conidium each; conidia of a characteristic shape, elongate, fusiform with a long appendage at the apex, attached by their broad end, and having a dark scar showing the point of attachment, 5–8 septate, dark brown, not constricted at the septa, thick-walled, with granular contents; the second or third cell from the base larger than the rest, conidia 46·8–95 × 10·4–18·2 μ; appendage hyaline, nonseptate, and slightly curved, longer than the body of the conidium, 49·4–127·5 μ; sclerotia develop profusely after 7–10 days in cultures in regular zones, cylindrical or somewhat irregular in shape, deep black or sooty black in colour, with numerous, stiff, hyaline bristles, 0·5–3·0 × 0·25–0·5 mm.

On leaves and pods of *Crotalaria verrucosa* L., Agricultural College Farm, Osmania University, Hyderabad-Dn., 22–4–1953, coll. M. A. Salam and P. N. Rao, specimen deposited in the Herb., 'Hy' Botany Dept., Osmania University, No. 11, and the type culture deposited in the Commonwealth Mycological Institute, Kew, No. 52821.

Trichoconis crotalariæ Salam & Rao sp. nov.

Maculæ irregulares, albidæ; mycelium bene evolutum, olivaceo-brunneum, 3·6–6 μ crassum ramosum ad maturitatum, septis positis 18–36 μ inter se distantibus. Rami efformat angulum rectum cum axi principali. Conidiophori haud clare separabilis ad hyphis, erecti, septati, apice tumescents, 52–156 × 3·6–6 μ, unum conidium ferentet singuli; conidia forma typica, prædita, elongata, fusiformia, apendice longa ad apicem ornata, basi latiore atque ornata cicatrice fusca monstrante partem fixationis, 5–8 septata fusce brunnea, haud constricta ad septa, crassis parietibus prædita, contentis granularibus, secund atque tertia cellula ex basi largiores caeteris; conidia 46·8–95 × 10·4–18·2 μ; apex fastigatur in apendicum tenuiter curgatum, 49·4–127·5 μ longum, 1–2·6 μ latum, longiorum quam ipsum conidium; sclerotia profuse evoluta post 7–10 dies in culturis in zonas regularies; in vitro sclerotia crescunt verticaliter, suntque cylindrica arque aliquantum irregularia figura, alte nigra vel fuliginose nigra colore, ornata plurimis pilis erectis, hyalinis, rigidis, magnitudinis 0·5–3·0 mm. longa atque 0·25–0·5 mm. lata.

Typus lectus in foliis atque fructibus *Crotalariæ verrucosæ* Linn. in Agricult. Coll. Farm, in Osmania Univeristy, in urbe Hyderabad-Dn. die 22 aprilis anni 1953, leg. M. A. Salam et P. N. Rao, et positis in Herb. 'Hy' Botany Dept., in Universitate Osmania No. 11, et in Commonwealth Mycological Institute, Kew, sub numero 52821.

Summary

A new species of *Trichoconis*, *T. crotalariæ* Salam and Rao is described. The fungus infects leaves and pods of *Crotalaria verrucosa* L., in the Agricultural College Farm, Hyderabad-Dn. It is compared with the two other species of the genus, *T. caudata* (App. & Str.) Clements and *T. padwickii* Ganguly from both of which it is found to differ in spore and sclerotial size and certain other characters.

Acknowledgments

We are grateful to Prof. M. Sayeeduddin for his encouragement during the investigations. Our thanks are also due to Prof. H. Santapau for the Latin translation of the diagnosis and to Dr. M. B. Ellis of the Commonwealth Mycological Institute, Kew, for his confirmation of the identification. We also wish to record our thanks to Prof. T. S. Sadasivan for his helpful suggestions.

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RUSTS OF HYDERABAD

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(Received for publication on June 2, 1954)

Introduction

A preliminary survey of the "Fungus Flora of Hyderabad" has been reported by S. Waheeduddin and the authors at the 41st Session of the Indian Science Congress. In that report a few species of rusts occurring on cereal plants have also been included. In this paper, the authors propose to record a few more members of the Uredinales. As far as the knowledge of the writers goes, there is no published record of the rusts from Hyderabad. The specimens have been deposited in the Botany Laboratory of the Osmania University.

Description

Dasturella divina (Syd.) Mundkur and Kheswalla

On the leaves of *Dendrocalamus strictus*, Nees. Mannanoor, coll. P. R. and M. A. S., December 1953. Since the material was collected late after the monsoon, the leaves showed only the presence of telia. Teliosori subepidermal, erumpent; teliospores fascicled into large, coloured, sessile heads without cysts. The teliospores do not become free; they remain fused both laterally and at either end to form flabelliform crustose heads. A thin hyphal layer envelopes the entire telial head. The teliosori are striate. Paraphyses are present as a marginal fringe. Though the collected rust specimen shows only the telial stage, the presence of the paraphyses gives a clue to the fact that the telia have developed within the old uredia as suggested by Thirumalachar *et al.* (1947). The teliospores are produced basipetally in chains. The alternate host for this parasite is *Randia dumetorum* (Thirumalachar *et al.*, 1947) which is very common in Hyderabad, but so far no aecia and pycnia have been noticed.

Puccinia leucadis Syd. (Sacc. *Sylloge Fungorum*, XVII: 329)

On the leaves of *Leucas linifolia*, Spreng., Aurangabad, coll. M. A. S., October 1953. Only the telial stage was found. Telia subepidermal. Teliospores are two-celled and pedicellate, pedicel hyaline. The spore measures $20{\cdot}8–33{\cdot}8 \times 15{\cdot}6–20{\cdot}2\,\mu$ long, $2{\cdot}6–5{\cdot}2\,\mu$ thick, Pedicel $20{\cdot}8–78\,\mu$ long.

Puccinia heterospora Berk. and Curt. (Sacc. *Sylloge Fungorum*, VII: 695)

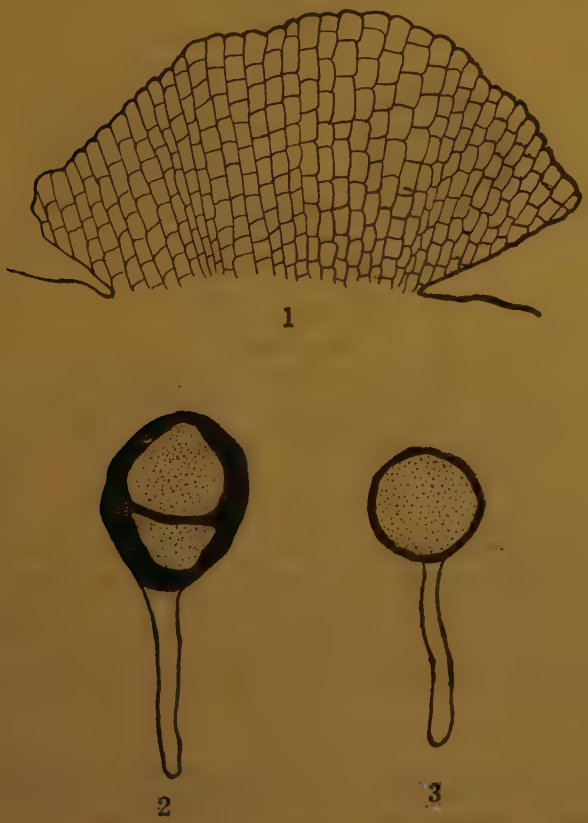
On the leaves and stems of *Sida spinosa*, Linn., Vikarabad, coll. P. R., November 1953. The infection was heavy. The affected areas

appear as dark-brown concentric patches. Only telia were present. Teliospores globose, chestnut-brown in colour, pedicellate; pedicel hyaline and much longer than the spore. The spores measure $20 \cdot 4 \times 17\ \mu$.

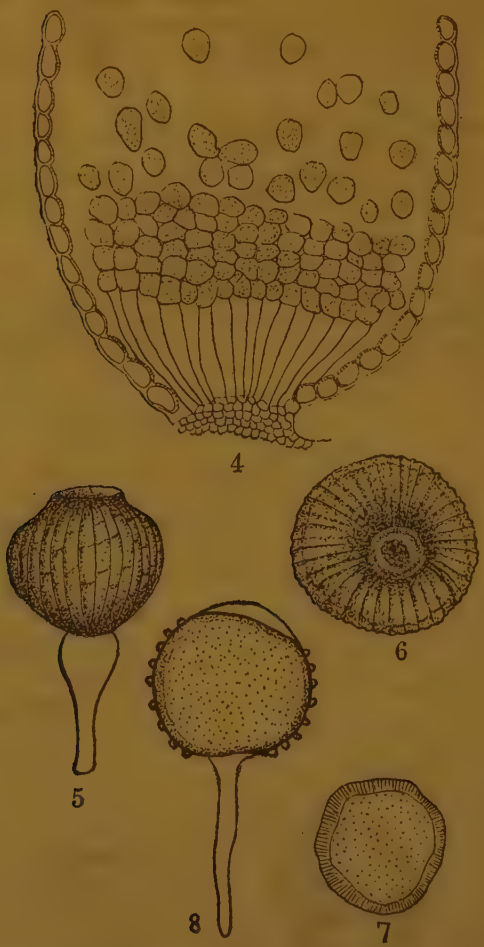
Puccinia penniseti Zimm. (Sacc., *Sylloge Fungorum*, XVII: 390)

On the leaves of *Pennisetum typhoides* Stapf. and Hubb., and *Solanum melongena* Linn. Aurangabad and Adigmet, coll. M. A. S. and P. R., November 1953.

The infection on the leaves of *Pennisetum typhoides* Stapf. and Hubb., showed the presence of the two spore forms, *viz.*, the uredia and the telia. The æcial stage has been described on *Solanum melongena* Linn. by Ramakrishnan and Soumini (1948). The authors



FIGS. 1–3. Fig. 1. Camera lucida drawings of the mature telium of *Dasturella divina*, × 180. Fig. 2. Teliospore of *Puccinia leucadis*, × 600. Fig. 3. Teliospore of *Puccinia heterospora*, × 660.



FIGS. 4–8. Transverse section through an æcium on *Solanum melongena* showing a distinct peridium and chains of spores formed on a fertile layer, × 180. Fig. 5. Teleutospore of *Trochodium sampathense* showing the characteristic inflation of the stalk, × 660. Fig. 6. Surface view of the teliospore of *Trochodium sampathense* showing the central pit and ribs on the exospore, × 660. Fig. 7. Aeciospore of *Trochodium sampathense*, × 660. Fig. 8. Teliospore of *Uromyces blainvelleæ*, × 660.

collected heavily infected leaves of *Solanum melongena* Linn. showing beautiful, large, salmon-orange, hyphophyllous, columnar æcidial cups arranged in irregular concentric circles, projecting beyond the surface of the leaf. A section through the æcium showed a distinct peridium. The peridium consists of polygonal cells prominently verrucose on the inner surface. The æciospores were catenulate, formed

on a prominent fertile layer of elongated cells. The spores are globose to angular, yellowish orange, thin-walled and measure $20 \times 18\,\mu$.

Trochodium sampathense Thirumalachar (*J. Indian bot. Soc.*, 21: 202)

On the leaves of *Argyreia cymosa* Sweet. Adigmet, coll. M. A. S. and P. R., November 1953. The infection shows the presence of all the three spore forms, *viz.*, Telia, æcia and pycnia. The teliospores show the characteristic inflation at the place of attachment, and this is smaller in size than the spore. The exospore is thick with longitudinal striæ, which radiate from the apex and converge towards the base. Teliospores are deep brown in colour and uninucleate. They measure $21 \cdot 5$–$25 \cdot 7\,\mu$. The spores are almost spherical, with a rounded apex. The margin of the spore is conspicuously thickened and thickening is not uniform throughout. The pycnia are flask shaped, sunken in the host tissue with an ostiole and paraphyses. Aeciospores are yellow and polyhedral, measuring $20 \times 17\,\mu$.

Uromyces blainvilleæ, Berk. (Sacc. *Sylloge Fungorum*, VII: 576).

On the leaves and stem of *Blainvillea rhomboidea*, Cass. (*B. latifolia*), Vikarabad, coll. P. R., November 1953. Only telia were present. They appear as black concentric patches on the leaves and stem which can be identified macroscopically. Telia hypophyllus. Teliospores globular, measuring $29 \cdot 7 \times 27 \cdot 2\,\mu$, pedicellate; pedicel hyaline, $23 \cdot 8\,\mu$ long. Each spore has a characteristic apical orientation, measuring $3 \cdot 4 \times 17 \cdot 0\,\mu$ and hyaline. Teliospore verrucose and chestnut brown in colour.

Summary

In this paper six species of rusts, *viz.*, *Dasturella divina* (Syd.) Mundkur and Kheswalla, *Puccinia leucadis* Syd., *Puccinia heterospora* Berk. and Curt., *Puccinia penniseti* Zimm., *Trochodium sampathense* Thirumalachar, and *Uromyces blainvilleæ* Berk., occurring on common angiospermic plants have been reported for the first time from Hyderabad.

Acknowledgments

The authors wish to express their gratitude to Prof. M. Sayeeduddin, Head of the Department of Botany and Principal, University Science College, for his keen interest during this investigation.

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* Not seen in original.

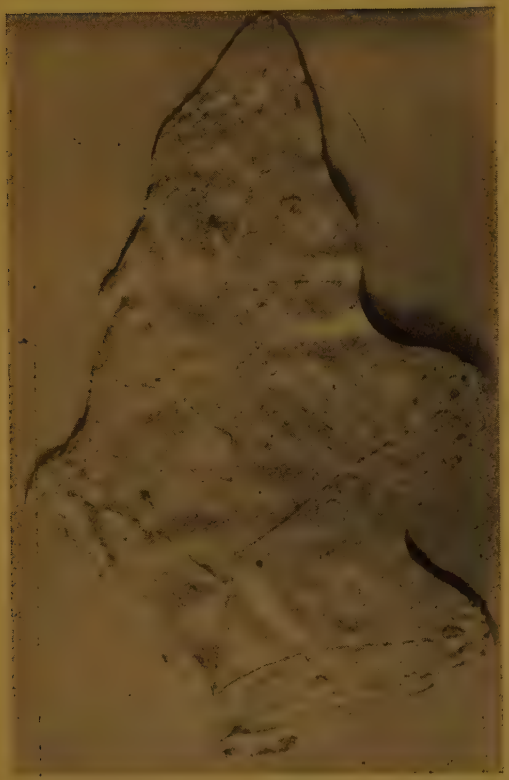
EXPLANATION OF PLATE

A. *Sida spinosa* Linn. Showing the telia of *Puccinia heterospora*. Natural size. B, *Blainvillea rhomboidea* Cass., showing the telia of *Uromyces blainvilleæ*. Natural size. C, *Solanum melongena* Linn., showing the æcidial cups of *Puccinia penniseti*. Half the natural size. *D. Argyreia cymosa* Sweet showing the telia of *Trochodium sampathense*. Natural size. E, Leaf of *Dendrocalamus strictus* Nees, showing the striate telia of *Dasturella divina*. Natural size.

A

B

P. Ramachar and M. A. Salam



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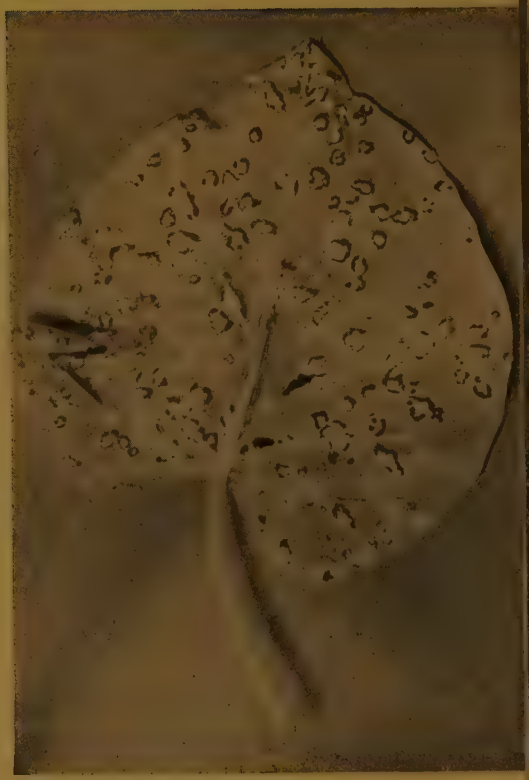

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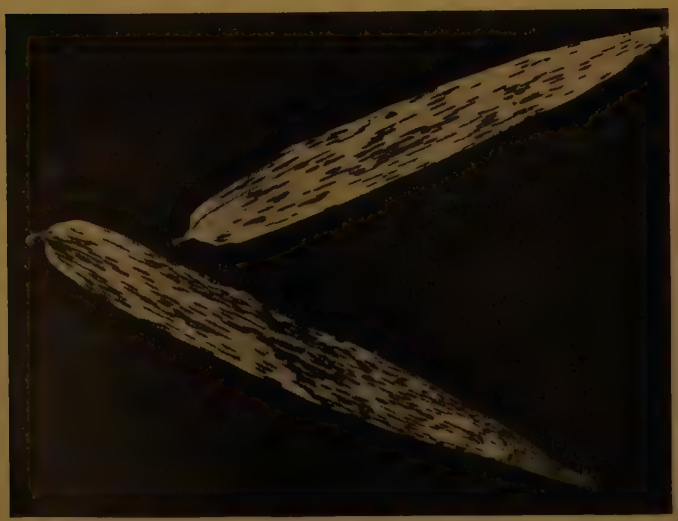

E

P. Ramachar and M. A. Salam

SOIL CONDITIONS AND ROOT DISEASES

XII. The Role of Zinc and Manganese in Altering Host Metabolism

By R. Kalyanasundaram

University Botany Laboratory, Madras—5

(Received for publication on May 25, 1954)

Introduction

Experimental evidence by many workers indicates that susceptibility of plants to diseases, particularly to attacks by root disease pathogens, is conditioned to a considerable extent by their nutrition (Walker and Hooker, 1945; Walker and Foster, 1946 and Garrett, 1948). Control of wilt diseases caused by vascular Fusaria has been reported by amending the naturally infested soils with trace elements, especially Zinc and Manganese (Sarojini, 1951; Yogeswari, 1950 and Sulochana, 1952). Recent work of Gäumann (1951) with wilt toxins, has also indicated that the degree of damage caused by these toxins depends on the nutritional status of the host—the under-nourished being the most affected.

Working with the host metabolism of *Fusarium vasinfectum* infected cotton plants, the author (Kalyanasundaram, 1953) found that the two strains of *Gossypium hirsutum* (Cambodia and Madras Uganda) which are naturally resistant to the wilt disease caused by *F. vasinfectum*, have more of reserve food materials in their shoot system as indicated by the accumulation of ascorbic acid and carbohydrates, compared to the two strains of *Gossypium arboreum* (Karunganni 2 and Malvi 9) susceptible to the disease.

The present investigation was undertaken to understand the metabolic status of cotton plants (*G. arboreum*) susceptible to the disease caused by *F. vasinfectum*, as affected by Zinc and Manganese nutrition.

Materials and Methods

Susceptible strain cotton plants, Karunganni 2, were grown in glazed earthenware pots containing garden soil amended with Zn and Mn at the optimum level of 100 p.p.m. (Sulochana, 1952). Estimations of ascorbic acid, total carbohydrates and reducing sugars were made in the shoots of these plants following standard methods (Kalyanasundaram, 1953), at intervals of five days up to the 40th day of plant growth, both "per gram" basis and "per plant" basis. The dry weights of the shoots of plants in all the series were also recorded.

Experimental Results

The results are presented in Figs. 1 and 2.

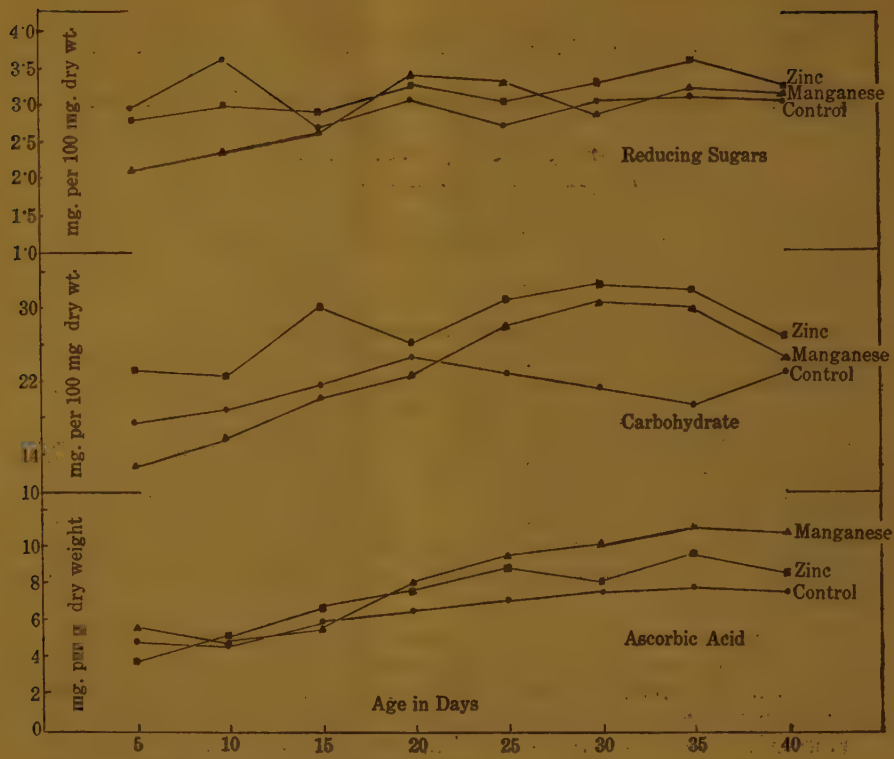

FIG. 1. Graphs showing the ascorbic acid, carbohydrate and reducing sugar contents of cotton plants grown in soil amended with Zinc and Manganese. Results expressed 'per gram' basis.

Ascorbic acid

Although plants growing in the Mn amended soil have more ascorbic acid synthesised, as indicated by the higher content per gram of leaf tissue, the absolute quantity of ascorbic acid per plant was significantly higher only in plants growing in the Zn amended soils from the 15th day.

Carbohydrate

The percentage carbohydrate in plants growing in Mn and Zn amended soils was not significantly higher than the control plants. But the total carbohydrate content per plant in the Zn amended soils alone was highest from the 15th day.

Reducing sugar

The quantity of reducing sugar per plant in the Zn amended soils was greater than the plants in the Mn amended soils and control.

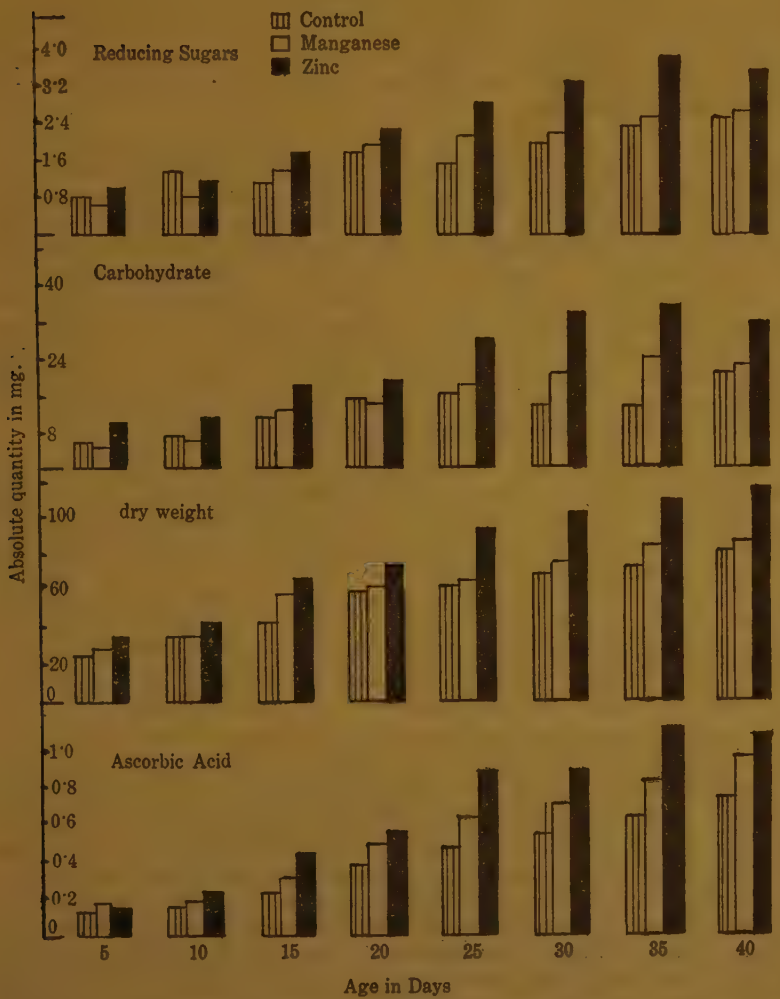

FIG. 2. Histograms showing ascorbic acid, carbohydrate, reducing sugar contents and the dry weight of shoots of cotton plants grown in soil amended with Zinc and Manganese. Results expressed 'per plant' basis.

Dry weight of shoots

Significantly higher dry weights were seen in plants with Zn nutrition especially from the 15th day than in plants with Mn nutrition.

DISCUSSION

Among the trace elements, Manganese (Rudra, 1938 and Rangnekar, 1946) and Molybdenum (Hewitt *et al.*, 1950) seem to be beneficial for the synthesis of ascorbic acid in plants. Although Manganese

in the present experiment tended to increase the amount of ascorbic acid synthesised as shown by higher ascorbic acid per gram of leaf tissue (Fig. 1) did not increase the absolute quantity per plant (Fig. 2). The absolute quantity of ascorbic acid per plant was highest in Zn treated plants (Fig. 2).

Recent work of Brown and Wilson (1952) indicated that Zn is necessary for the growth, especially of Asiatic cotton (*G. arboreum*). Since higher dry weight has been correlated with increased ascorbic acid (Reid, 1942) and as Zn considerably increased the dry weight of the tops of these treated plants, there was significantly higher ascorbic acid in Zn treated plants (Fig. 2). In this connection it is worthwhile mentioning the work of Virtanen (1936) who established the essentiality of ascorbic acid for plant growth, which he calls as a "phytohormone". The increased carbohydrate and reducing sugar content of Zn treated plants is a natural corollary of increased ascorbic acid, as it seems to be involved directly in the photosynthetic formation of formaldehyde (Baur and Fricker, 1937).

Earlier work of the author (Kalyanasundaram, 1953) on host metabolism in relation to wilt diseases indicated that the two strains of cotton (*G. hirsutum*), which are naturally resistant to the disease caused by *F. vasinfectum*, have a very high reserve of ascorbic acid and carbohydrate compared to the two susceptible strains (*G. arboreum*). The increased ascorbic acid and carbohydrate content of Zn treated susceptible strain of cotton plant (*G. arboreum*) reported here is significant in the light of the work of Sulochana (1952) who reported increased resistance to wilt of cotton caused by *F. vasinfectum* with Zn amendment to wilt infested soils.

It was shown by the author (Kalyanasundaram, 1953) that plants of *G. hirsutum* and *G. arboreum* infected by *F. vasinfectum* (plants of *G. hirsutum* are not resistant to infection but resistant to wilt only) showed a decrease in ascorbic aicd and carbohydrate following the infection period. But the resistant strain, in spite of the decrease following infection, had a higher reserve of ascorbic acid and carbohydrate than the normal plants of the susceptible strain. This change or shift in the metabolism of the infected resistant strains was interpreted as a defensive mechanism to ward off the disease. Similar metabolic changes have been reported by Keyworth and Dimond (1952) and Davis and Dimond (1952), in wilt of tomato caused by *F. lycopersici*, where resistance was induced by physical or chemical treatments to infected plants.

It will not be out of place to cite here the work of Brown and Wilson (1952) dealing with effects of Zn on several species of cotton. They indicated that the Asiatic cottons (*G. arboreum*) were deficient to Zinc unlike the American cottons (*G. hirsutum*). Brown and Wilson, on the basis of earlier work of Skoog (1940) and Tsui (1948), interpreted that the absence of Zinc reduced the auxin levels in both the species of cotton, but that in some way the American cotton plants were able to use whatever auxin that remained more effectively than the plants of Asiatic type.

Thus the addition of Zinc to soils seems to have created condition of plant growth in which the susceptible cotton (*G. arboreum*) almost simulates the resistant strains of cotton as far as their ascorbic acid and carbohydrate metabolism is concerned and how far this condition is responsible for inducing the resistance to wilt remains to be worked out.

The resistance acquired by the wilt-susceptible cotton plants given Zn nutrition is most probably due to a change in the physiological behaviour of living tissues of the plants working probably by way of the growth producing auxin in the presence of Zinc. The evidence to this is given by the increased ascorbic acid, a phytohormone, in the Zn treated plants (Fig. 2). This induced character, however, is naturally present in the normal resistant cotton (*G. hirsutum*) and may explain its natural resistance to wilt disease caused by *F. vasinfectum* (Kalyanasundaram, 1953).

The above hypothesis does not rule out any other possible resistance mechanism associated with the naturally resistant and artificially resistant plants. A study of the auxin levels and enzymatic status of these hosts attacked by *F. vasinfectum* may well help us to understand how exactly these metabolic changes reported here, favourable in warding off the disease, are achieved.

Summary

1. The wilt susceptible variety of cotton plants (*Gossypium arboreum*) has a higher reserve of ascorbic acid, carbohydrate and reducing sugars when grown in soil amended with Zinc.

2. The cotton plants grown in Zinc treated soil, have their ascorbic acid and carbohydrate metabolism similar to that of the wilt resistant strain of cotton (*Gossypium hirsutum*) reported elsewhere, and this may be responsible for the resistance acquired by Zinc treated plants reported earlier by Sulochana (1952).

3. Since Zinc is known to be responsible for the production of growth producing auxin (Tsui, 1948) and also very essential for the growth of Asiatic cotton (*G. arboreum*) than American cotton (*G. hirsutum*), the higher reserve of Vitamin C and carbohydrate in Zinc treated Asiatic cotton (*G. arboreum*) may be due to good plant growth in the presence of auxin(s) induced by the Zinc amendment.

Acknowledgments

The writer is grateful to Professor T. S. Sadasivan and Dr. C. V. Subramanian for criticism and suggestions in the preparation of this paper and to the Government of India for the award of a scholarship.

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ALPAKESA, A NEW GENUS OF THE SPHÆROPSIDALES

By C. V. Subramanian and K. Ramakrishnan
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(Received on July 7, 1954)

In the course of a taxonomic study of the genus *Neottiospora* Desm., we had the opportunity of examining a fragment of the type specimen of *N. yuccæfolia* Hall (Hall, 1915) made available to us through the kindness of Dr. D. P. Rogers of the New York Botanic Garden and Dr. J. A. Stevenson of the Herbarium of the United States Department of Agriculture. The type collection of this species is on leaves of *Yucca* collected from Pullman, Washington, U.S.A.

The fungus forms minute black erumpent pycnidia scattered on both surfaces of leaves. The pycnidia are separate, not immersed in a stroma, spherical and are ostiolate. The pycnidial wall is membranous and made up of two to three layers of pale brown, polygonal cells. The conidia are produced singly on short, stout, hyaline, non-septate, cylindrical, closely packed conidiophores covering the inner surface of the pycnidial wall. The conidia are hyaline, one-celled, nearly cylindrical, straight or slightly curved, with slightly rounded or flat base and with two to six simple, unbranched, filamentous, non-septate appendages arising from several points on or near the rounded apex of the conidium and of about the same length. The conidia are $39 \cdot 8 \times 8 \cdot 3$ $(33\text{–}65 \times 4 \cdot 9\text{–}13 \cdot 3)\,\mu$ in size; the appendages are $33\text{–}41 \cdot 5 \times 0 \cdot 8\,\mu$ in size.

A study of *Neottiospora caricum* Desm., the type species of the genus, has indicated that in *Neottiospora*, there is only one appendage for the spore and it is apical; and that the appendage is mucoid and evanescent and is in the form of an inverted hollow cone with hyaline, thin walls (Subramanian and Ramakrishnan, 1953). Considering, therefore, the true nature of the spore-appendage in this genus, as brought out by a study of its type species, it becomes evident that *N. yuccæfolia* Hall is not congeneric with the type species of *Neottiospora*. In *N. yuccæfolia* the conidia have more than one appendage and they are, unlike those of *N. caricum*, persistent, non-mucoid and filamentous. *N. yuccæfolia* is, therefore, not a *Neottiospora*.

So far as we are aware, no other genus of the Sphæropsidales known can accommodate this fungus. We therefore propose a new genus for it. The generic name ***Alpakesa*** is derived from Sanskrit: *Alpa* (अल्प) = few and *kēsa* (केश) = hair, indicating the few appendages of the conidia.

FIG. 1. *Alpakesa yuccæfolia*.—Conidia from type specimen, Herb. M.U.B.L. No. 699.

Alpakesa Subramanian and Ramakrishnan gen. nov.

Fungus Imperfectus, Sphæropsidales, Sphærioideæ, Hyalosporæ.

Pycnidia black, separate, membranous, non-stromatic, ostiolate. Conidiophores simple, hyaline, lining the pycnidial cavity. Conidia acrogenous, hyaline, one-celled, with two or more filamentous, unbranched, non-septate, hyaline, persistent, non-mucoid appendages arising from several points on or near the apex.

Pertinet ad Fungos Imperfectos, ad Sphæropsidales, Sphærioideas, Hyalosporas.

Pycnidia nigra, separata membranacea, non-stromatica, ostiolata. Conidiophori simplices, hyalini, operientes internam superficiem cavitatis pycnidialis. Conidia acrogena, hyalina, 1-cellulata, ornata duobus vel pluribus appendicibus filamentosis, haud-ramosis, haud-septatis, hyalinis, persistentibus, haud-mucoideis, surgentibus e pluribus locis in apice vel apici proximis.

Species typica:

Alpakesa yuccaefolia (Hall) Subramanian and Ramakrishnan comb. nov.

Basinym: *Neottiospora yuccæfolia* Hall in *Phytopathology*, 5: 57 ic., 1915, Saccardo, *Sylloge Fungorum*, 25: 184, 1931.

Type specimen: on dead and dying leaves of *Yucca*, Pullman, Washington, June 1, 1914, coll. John G. Hall, Herb. M.U.B.L. No. 699 ex Herb. N.Y. bot Garden (Fungi of Washington); Herb. M.U.B.L. No. 709 ex Herb. U.S.D.A.

We are grateful to Dr. D. P. Rogers and Dr. J. A Stevenson for specimens. We thank Prof. H. Santapau for the Latin diagnosis and Dr. V. Raghavan for suggesting the generic name derived from Sanskrit.

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A CONTRIBUTION TO THE EMBRYOLOGY OF THE PHYTOLACCACEÆ

II. Fertilization and the Development of Embryo, Seed and Fruit in *Rivina humilis* Linn. and *Phytolacca dioica* Linn.

By L. B. Kajale

Mahakoshal Mahavidyalaya, Jabalpur, M.P.

(Received for publication on February 20, 1954)

The present communication on the embryology of the Phytolaccaceæ deals with fertilization and the development of the embryo, endosperm, seed coat and fruit in *Rivina humilis* Linn. and *Phytolacca dioica* Linn. The former has been studied in greater detail and the account of the development of the fruit and the structure of the mature seed is based entirely on the study of this species.

The previous literature on the subject has been summarized in the first paper (Kajale, 1944). The only notable work published since then is that of Johansen (1950) who from the descriptions of Lewis (1905) and Mauritzon (1934), has inferred that the embryo development in *Phytolacca decandra* and *Rivina humilis* agrees closely with the *Myosurus*-variation of the Onagrad-type.

Material and Methods

The material of *Rivina humilis* was collected from the Empress Gardens, Poona. *Phytolacca dioica* was obtained from the Indian Botanical Garden, Calcutta. It was collected by Prof. J. Venkateshwarlu of the Andhra University. Formalin-acetic-alcohol was used as the fixing reagent in both cases. Dehydration and embedding was done according to the customary methods. Sections were cut 10–12 μ thick. Heidenhain's and Delafield's hæmatoxylins were used for staining. The latter proved more satisfactory for the early stages of embryo development. The structure of the mature seed was studied from free hand sections.

Fertilization

The pollen grains reach the stigma at the three-celled stage. There they germinate. The pollen tube makes its way through the style to the micropyle. By this time the cells of the nucellus situated directly below the micropyle become radially elongated. These cells which are slightly richer in cytoplasm than the surrounding cells form 14–17 layers in *Rivina humilis* and 7–10 layers in *Phytolacca dioica*. On the sides of the embryo-sac in both the species there are 4–6 layers of cells at this stage. The change in the cells at the micropylar end is more pronounced in *Rivina humilis* than in *Phytolacca dioica*. Such a radial stretching of the nucellar cells is known to occur in several allied families

like the Chenopodiaceæ (Mahabale and Solanky, 1953), Amaranthaceæ (Kajale, 1940 *b*), Ficoidaceæ (Kajale, 1940 *a*), Molluginaceæ (Joshi and Rao, 1936) and Portulacaceæ (Kajale, 1942).

The pollen tube after penetrating the micropyle passes through the inter-cellular spaces between elongated cells which probably have a two-fold function to perform. In the first place, by their radial elongation, they provide an easy passage for the advancing pollen tube. Secondly, as indicated by their cytoplasmic contents, they seem to supply the necessary nourishment to the pollen tube.

On entering the embryo-sac, the pollen tube passes in between the two synergids and the egg, generally destroying only one of the synergids (Fig. 1). The other synergid degenerates soon afterwards (Fig. 2), but frequently it persists for some time inside the embryo-sac and can be seen during the early stages of embryo development (Fig. 5). Rarely both the synergids persist for some time during post-fertilization stages. Such persistent synergids were observed both in *Rivina humilis* and *Phytolacca dioica*. No change was noted in their structure during this time (Fig. 4). As previously reported by Joshi (1936) the synergids have beaks. They also show the presence of hooks (Figs. 1 and 4).

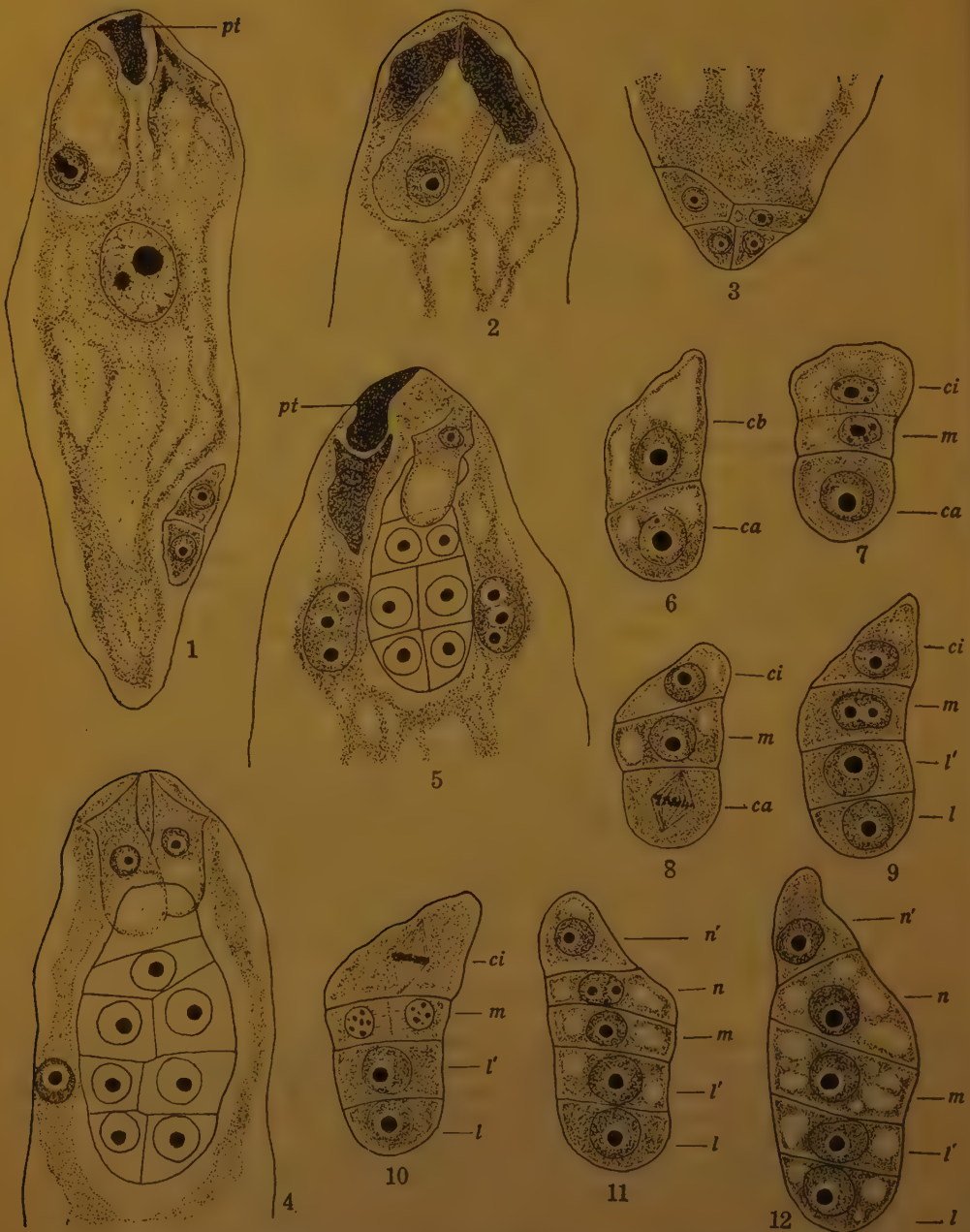
Double fertilization proceeds according to the normal schedule (Fig. 1). The degenerating pollen tubes in both the species persist inside the embryo-sac for some time after fertilization and can be seen during the early stages of embryo development (Fig. 5).

The behaviour of the antipodals in *Rivina humilis* calls for some remarks. The antipodals are generally three in number and according to Mauritzon (1934) they degenerate about the time of fertilization. The author, however, has seen that some times they increase in number (Fig. 3). As many as six antipodals were counted in some cases. They may also persist for a while after fertilization and become laterally placed as reported by the author (Kajale, 1940 *b*) in several members of the Amaranthaceæ (Fig. 1). However, sooner or later the antipodals degenerate and no trace of them is seen during the early stages of embryo development.

Numerous starch grains are deposited in the mature embryo-sac of both the species.

Obturator

The author (Kajale, 1944) has previously described the development of long hair-like outgrowths from the funiculus near the micropyle in *Phytolacca dioica*. This may be described as an obturator representing the end of the transmitting tissue. A similar but much reduced tissue is also developed in *Rivina humilis*. It consists of a group of enlarged cells developing at the base of the ovule near the micropyle (Fig. 55). It differs from that of *Phytolacca dioica* in that it develops from the wall of the ovary while in *Phytolacca* it is an outgrowth of the funiculus. The obturator in *Rivina* persists for some time after fertilization.



FIGS. 1–12. *Rivina humilis*.—Fig. 1. An embryo sac showing double fertilization, remains of the pollen tube (*pt*), a degenerating synergid and two laterally

placed antipodals. The other synergid and one of the antipodals are not shown. Fig. 2. Micropylar part of the embryo sac showing egg and two degenerating synergids. Fig. 3. The chalazal end of the embryo sac showing four antipodals. Fig. 4. Micropylar part of an embryo sac showing two persisting synergids and embryo. Fig. 5. The same showing embryo, one persisting and another degenerating synergid, remains of the pollen tube (*pt*) and two endosperm nuclei, each with three nucleoli. Figs. 6–12. Various stages in the development of proembryo. Note the variation in the vacuolation of proembryonic cells. × 750.

Embryo

A few stages in the embryo development have been described by Lewis (1905) in *Phytolacca decandra* and by Mauritzon (1934) for *Rivina humilis* and three other species of the Phytolaccaceæ, but these do not give a correct idea about the embryo development in the family. In the present communication, therefore, an attempt has been made to work out this phase of the life-history in a thorough manner. The earlier stages particularly have been critically studied both in *Phytolacca dioica* and *Rivina humilis*.

The oospore divides by a transverse wall into two cells, the terminal cell *ca* and the basal cell *cb* (Figs. 6 and 28). The basal cell *cb* divides next transversely forming the cells *m* and *ci* (Figs. 7 and 29). The division of the cell *cb* may sometimes be oblique. Shortly afterwards the cell *ca* also divides transversely forming the cells *l* and *l'* in *Rivina humilis* and *cc* and *cd* in *Phytolacca dioica* (Figs. 8, 9 and 29–31). The cell *ci* divides further in a transverse plane giving rise to two cells *n* and *n'* (Figs. 10 and 11). Sometimes the division of the cell *ci* is also in an oblique plane like the division of cell *cb* (Fig. 12). The cell *ci* in *Rivina humilis* divides transversely either before or after the appearance of the first vertical wall in the cell *m* or the division of this cell may synchronize with the first vertical division in *m* (Figs. 10, 11, 13 and 15). In *Phytolacca dioica* generally the cell *ci* divides transversely before any vertical division takes place in the proembryo (Fig. 31).

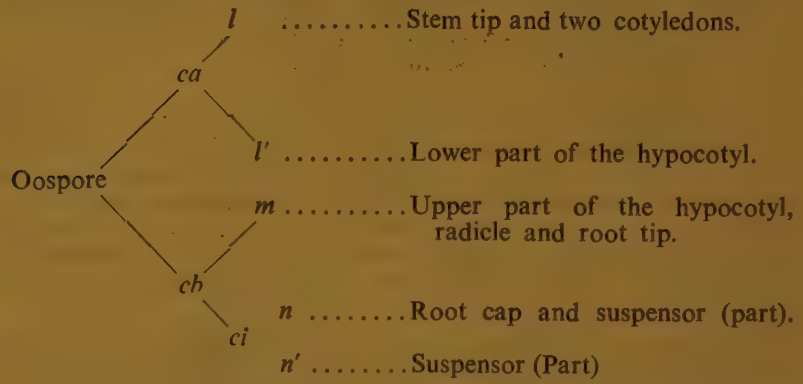
In this manner a linear proembryo of four or five cells is organized both in *Rivina humilis* and *Phytolacca dioica*. Mauritzon (1934) has also figured a proembryo of four or five cells in *Rivina humilis*, while Lewis (1905) has mentioned that in *Phytolacca decandra* the proembryo consists of five cells only. In *Petiveria alliacea* (Mauritzon, 1934), the proembryo consists of seven to nine cells before any longitudinal wall appears.

Though the derivation of the proembryonic cells is similar in both the species under investigation, the number of cells taking part in the formation of the embryo proper differs in the two plants. In *Rivina humilis*, three cells situated towards the apex of the proembryo develop into the embryo proper, while only two cells engender the entire embryo excluding the suspensor in *Phytolacca dioica*.

The origin of the various parts of the embryo in the two species is as follows:—

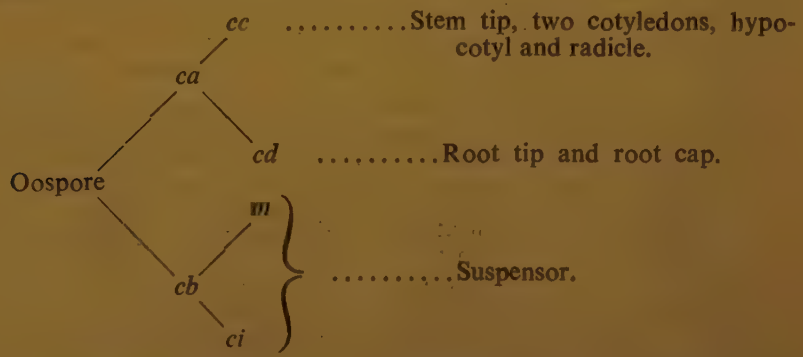
The apical cell (*l*) in *Rivina humilis* develops into the plumule and two cotyledons, the penultimate cell (*l'*) forms the lower part of the

hypocotyl. The third cell (*m*) completes the radicle including the root tip and the upper part of the hypocotyl. The cell *n* develops into the root cap and a part of the suspensor. The remaining part of the suspensor is formed by the cell *n'*. This is schematically shown below:—



Rivina humilis—A diagrammatic representation of the development of the embryo.

The destination of the various cells of the proembryo in *Phytolacca dioica* is quite different from *Rivina humilis*. The apical cell (*cc*) gives rise to the stem tip, two cotyledons, hypocotyl and radicle. The root tip and root cap are completed by the upper cell (*cd*) while the remaining cells (*m* and *ci*) develop into the suspensor as represented below:—



Phytolacca dioica—A diagrammatic representation of the development of the embryo.

Thus the derivation of the proembryonic cells and their destinations to form the various parts of the embryo leave no doubt that the embryo development in *Rivina humilis* does not at all agree with the *Myosurus*-variation of the Onagrad-type as inferred by Johansen (1950). Neither such a course of embryo development is met with in *Phytolacca dioica*. On the other hand the embryo development in *Rivina humilis* and

Phytolacca dioica conforms to the Chenopodiad and Caryophyllad-type respectively of Johansen (1950). In the light of the present work the case of *Phytolacca decandra* (Lewis, 1905) conforming to Myosurus-variation of the Onagrad-type (Johansen, 1950) appears doubtful and needs re-investigation.

The following table gives a comparative idea about the embryonomic formulæ in respect of the origin, disposition, eventual destinations and the histogenic functions of the proembryonic cells of *Rivina humilis* and *Phytolacca dioica* during the three successive generations. Abbreviations are after Johansen (1950).

Rivina humilis	*Phytolacca dioica*
1st cell generation; $ca = pco + pvt + phy$ (part). $cb = phy$ (part) $+ co + s + icc + iec.$	1st cell generation; $ca = pco + pvt + phy + co + icc + iec.$ $cb = s.$
2nd cell generation; the proembryo consists of four cells arranged in four tiers and their destinations are $l = pco + pvt.$ $l' = phy$ (part) $m = phy$ (part) $+ icc + iec.$ $ci = co + s.$	2nd cell generation; the proembryo consists of four cells arranged in four tiers and their destinations are $cc = pco + pvt + phy + icc.$ $cd = co + iec.$ $m = s$ (part). $ci = s$ (part).
3rd cell generation; the tiers l, l' and m have divided vertically in acropetal order and cell ci divides transversely into n and n'. There are thus eight cells disposed in five tiers with the same destinations as in the second cell generation except that $n = co + s$ (part) and $n' = s$ (part).	3rd cell generation; the destinations of the proembryonic cells are the same as in the 2nd cell generation.

Soon after the organization of the four- or five-celled proembryo, longitudinal divisions begin to appear. First such a division in *Rivina humilis* appears in the third cell (m) from the apex (Fig. 10). Next it appears in the penultimate cell (l') and finally in the apical cell (l) (Fig. 13). It will be seen from Fig. 14 that the planes of vertical divisions in these three cells are generally at right angles to each other. Occasionally, however, any two adjoining cells may divide in the same vertical plane (Fig. 13). The sequence of the longitudinal divisions in *Phytolacca dioica* could not be followed due to lack of suitable material.

After the completion of the vertical walls in all the three apical cells, quadrant formation begins. In *Rivina humilis* it takes place in an acropetal order starting in the third cell (m) from the apex (Fig. 10). Next it is completed in the cell l' and finally in the apical cell l (Figs. 13

FIGS. 13–25. *Rivina humilis*. Various stages in the development of embryo. For further explanation see text, × 750.

and 14). The embryo at this stage consists of 14–15 cells disposed in five or six tiers. The three apical tiers have four cells each. The remaining two or three micropylar tiers consist of one cell each or some may consist of two cells (Figs. 16 and 17). During quadrant formation the nuclei in a tier may or may not divide simultaneously (Figs. 16 and 18). As soon as quadrants are completed in all the three apical cells the dermatogen begins to differentiate in the tier formed by the cell *l'* in which the periclinal walls are first laid (Figs. 18 to 21). The periclinal walls next appear in the other two tiers on either side and are generally completed last in the apical tier (Figs. 23 to 25).

In *Phytolacca dioica*, the tier *cc* divides by two vertical walls and forms four cells designated together as *q*. The tier *cd* likewise forms four cells. Each cell of the quadrant (*q*) then divides transversely forming an octant stage (Fig. 32). In this respect the embryo development in *Phytolacca dioica* differs markedly from that in *Rivina humilis*. The differentiation of the dermatogen in the former is strictly in an acropetal order unlike the latter. The first periclinal wall delimiting the dermatogen appears in the cells of the tier *cd*. Later on it is completed in the cells of the octant formed from *q* (Figs. 33 and 34).

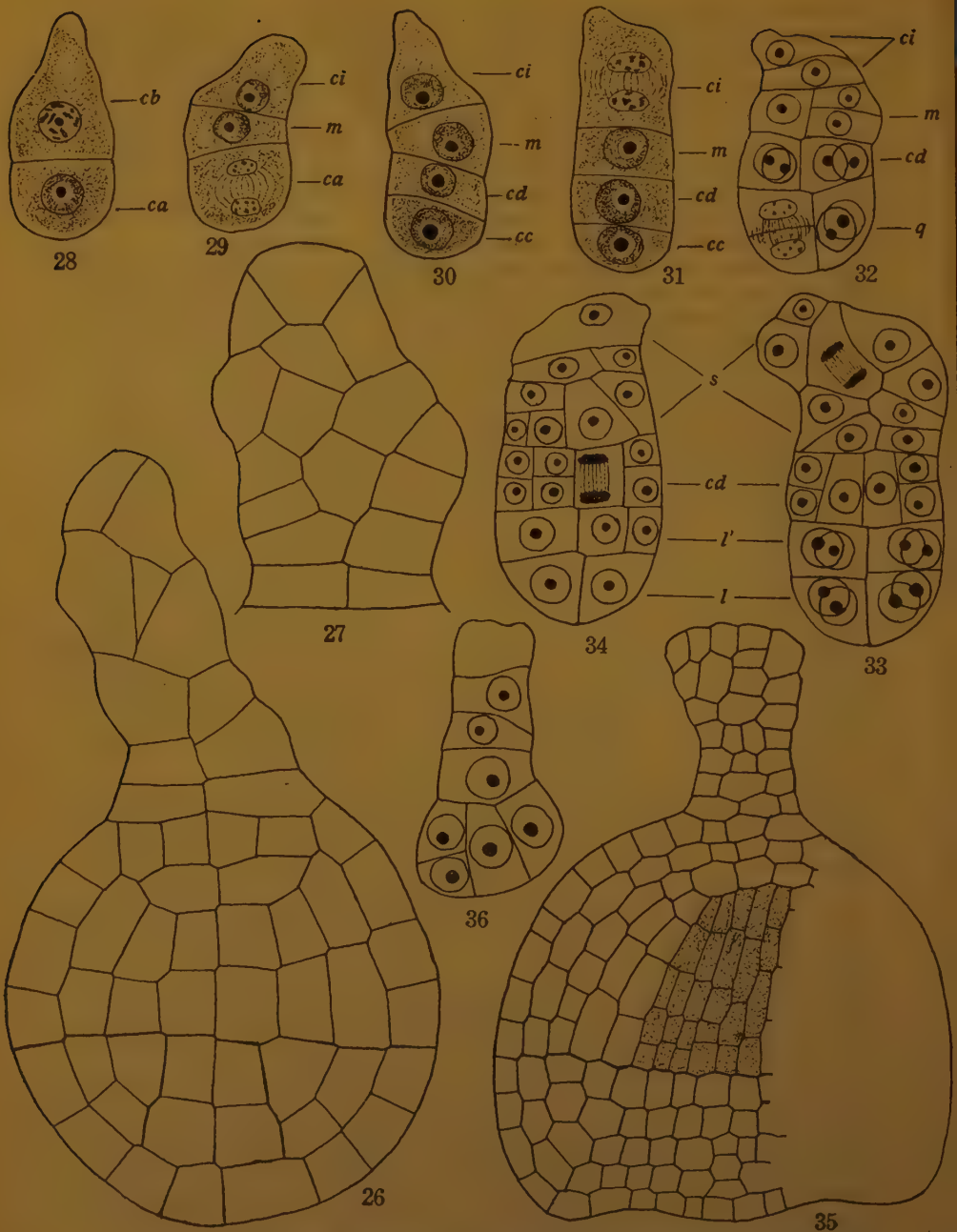
The deatils of the development of these tiers are given below:—

All the four cells of the apical tier (*l*) in *Rivina humilis* generally divide almost vertically and form four inner cells and four outer cells. These are designated by the letters *a* and *b* respectively (Figs. 22–24). Sometimes, however, the first division in the apical cell may be periclinal and then vertical (Fig. 23). The divisions in these cells may or may not be simultaneous. The four inner cells (*a*) then divide transversely forming four cells towards the apex and four cells towards the suspensor side. The cells which are cut off towards the apical side divide once again transversely and the outermost cells thus formed contribute to the dermatogen of the stem tip (Fig. 25).

The four outer cells (*b*) however, divide first periclinally and cut off the dermatogen (Fig. 25). After the completion of the dermatogen the cells of the apical tier, to begin with, divide mostly by vertical walls and ultimately by series of longitudinal and transverse divisions they develop into the two cotyledons and the plumule. A group of peripheral cells, one on each side, develops into the cotyledon, while the cells situated in the centre in between the two peripheral groups give rise to the plumule (Fig. 26).

The cell *l'* and the tier resulting therefrom in *Rivina humilis* divide, to begin with, mostly by longitudinal walls. During further development this tier produces the lower part of the hypocotyl.

In *Phytolacca dioica* the cell *cc*, as already stated, divides to form an octant (Figs. 32 and 33). The four apical cells of the octant (*l*) develop into the stem tip and two cotyledons while the upper four cells (*l'*) give rise to the hypocotyl and radicle in which periblem and plerome gradually differentiate. When the embryo becomes cordate, the periblem consists of two to three layers of cells towards the suspensor and about four layers of cells towards the apical side in both the species. The cells enclosed by the periblem form the plerome (Fig. 35).



FIGS. 26, 27 and 36. *Rivina humilis*.—Fig. 26. A stage in embryo development. Fig. 27. A suspensor. Fig. 36. An abnormal embryo. Figs. 28–35. *Phytolacca dioica*. Various stages in the development of embryo. For further explanation see text. Fig. 35, ×450; the rest, × 750.

The behaviour of the third cell (*m*) from the apex and the tier derived from it in *Rivina humilis* calls for some attention. The cell *m* is the first cell in the proembryo to divide longitudinally (Fig. 10). The second longitudinal division is also initiated in this very cell and occurs generally in a plane at right angle to the first (Fig. 15). As a result of these two divisions four cells are formed (Fig. 20). Each one of these cells divides transversely and eight cells are organized. These are arranged in two superposed tiers (*ma* and *mb*) of four cells each (Figs. 18 and 21). The organization of these two tiers, however, may not always follow the same sequence as described above and it might proceed along different lines discussed below. After the formation of the first vertical wall in the cell *m* the two juxtaposed cells instead of dividing vertically as in the normal cases might divide by transverse walls to form two tiers (*ma* and *mb*), each consisting of two cells (Fig. 22). Or the two juxtaposed cells may divide in two different planes at right angles to each other, *i.e.*, one dividing vertically and the other transversely (Figs. 16 and 17). The division of these cells also may or may not be simultaneous. However, in whatever manner the cells may divide the ultimate result is the formation of two superposed tiers (*ma* and *mb*) each consisting of four cells. Further there is no definite rule governing the formation of these two tiers in respect of time. They may be organized before the appearance of the walls delimiting the dermatogen or the organization of the two tiers may be delayed till the dermatogen is completed in the tier *l'* (Figs. 16–20).

In whatever way the two superposed tiers are formed, the tier *ma* which is adjacent to the embryonal mass forms by its further differentiation the periblem and plerome of the hypocotyl and radicle, while the tier *mb*, which is towards the side of suspensor, completes the root tip.

The tier *n* in *Rivina humilis* which consists of one cell in the beginning divides generally by two vertical and one transverse wall producing two superposed tiers *h* and *k* (Figs. 19 and 20). The tier *h* completes the root cap, while *k* forms a part of the suspensor.

In *Phytolacca dioica*, the cell *cd* as previously described forms a quadrant in which periclinal walls are cut off delimiting the dermatogen (Fig. 33). The cells of the dermatogen divide transversely. Transverse divisions are also initiated in the cells enclosed by the dermatogen and thus two tiers are organized (Fig. 34). The one towards the embryonal mass completes the root tip, while the other towards the suspensor side forms the root cap.

Suspensor

The suspensor in *Rivina humilis* is formed from the tiers *k*, *o* and *p*. In *Phytolacca dioica* it is developed from *m* and *ci*. Thus in the latter plant the cell *cb* and its derivatives form the suspensor only, while in the former species some of the derivatives of the cell *cb* contribute to the formation of the suspensor. The cells destined to form the suspensor begin to divide in different planes during the early stages

of embryo development (Figs. 19, 21, 24, 25 and 32–34). In both the species the suspensor becomes fairly massive particularly in the micropylar region, and in no part it remains uniseriate. Neither the cells of the suspensor become variously swollen as is the case in several variations of the Caryophyllad-type (Johansen, 1950). Two well-developed suspensors of *Rivina humilis* and *Phytolacca dioica* are shown in Figs. 27 and 35 respectively.

Mature Embryo

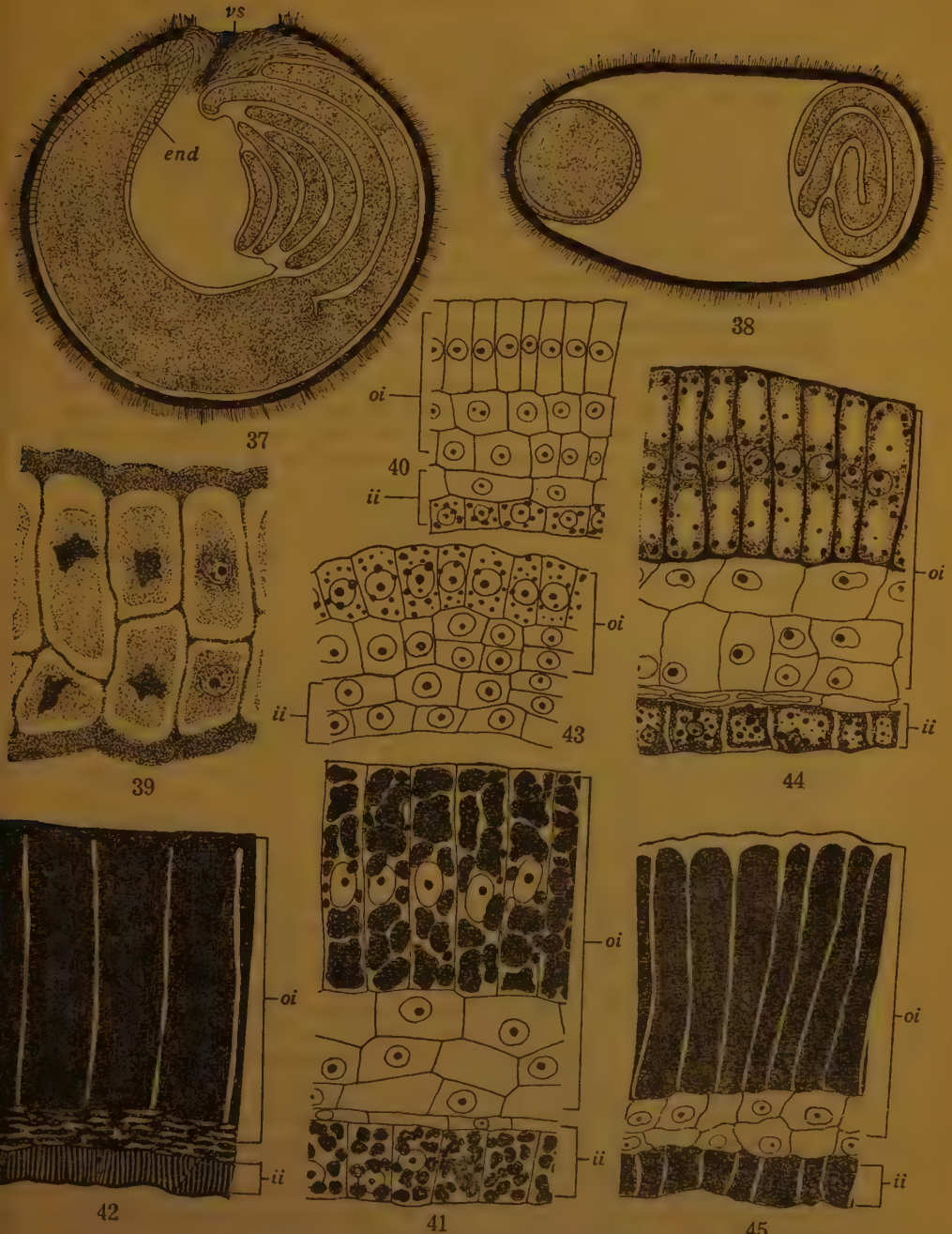
The structure of the mature embryo has been studied in *Rivina humilis* only. The mature embryo is dicotyledonous and annular. It encircles the perisperm as in the Centrospermales in general. The two cotyledons are not flat as figured by Engler and Prantl (1889, their Fig. 2 B), but are folded as shown in Fig. 38. Figure 37 gives a view of the embryo in longitudinal section. The cotyledons are lobed at the base as stated by Rendle (1925). The inner cotyledon slightly protrudes outside the fold of the outer cotyledon. Numerous starch grains are deposited in three histogenic layers of the embryo.

An Abnormal Embryo

A case of an abnormal embryo development was met with during the present investigation. This is shown in Fig. 36. The embryo consists of a row of four cells constituting the suspensor, at the apical end of which there is a small embryonal mass consisting of four cells. Obviously these cells have been formed by two vertical divisions of a single cell at the apex of the proembryo. A cell on the left has divided transversely. This embryo development mainly differs from the normal type in that only one cell has taken part in the formation of the embryonal mass.

Endosperm and Perisperm

The primary endosperm nucleus divides in a free nuclear fashion forming two to four in *Rivina humilis* and about sixteen nuclei in *Phytolacca dioica* before the oospore divides transversely. The endosperm nuclei continue to divide further and form a large number of nuclei in a free nuclear fashion. All these nuclei lie for some time in a peripheral layer of the cytoplasm. At about the stage of embryo development shown by Fig. 35 the wall formation begins in between the endosperm nuclei and the cells are organized. Each cell encloses a single nucleus. The wall formation starts in the micropylar region and finally extends right up to the chalazal end of the embryo-sac. The endosperm, therefore, becomes completely cellular throughout the embryo-sac consisting of large, thin-walled, vacuolated parenchyma cells. In this respect my observations agree with what Mauritzon (1934) has recorded in *Peteveria alliacea* and *Rivina brasiliensis*. Further the author agrees with Woodcock (1924) in that most of the endosperm is utilized by the embryo, while a small part of it is left in the form of a distinct cap over the radicle in the mature seed. The cap-like endosperm consists of about seven layers of cells over the tip of the radicle gradually decreasing to one cell in thickness towards the



FIGS. 37–42. *Rivina humilis*.—Fig. 37. L.S. of the seed. Fig. 38. T.S. of the seed. Fig. 39. A part of the endosperm in l.s. showing thickness of the peripheral tangential walls. Note the presence of the normal and degenerating nuclei. Figs. 40–42. L.S. of a part of the integuments showing stages in the development of testa. Figs. 43–45. *Phytolacca dioica*. The same as Figs. 40–42. *vs.*, vascular strand; *end.*, endosperm; *ii.*, inner integument. *oi.*, outer integument. Figs. 37 and 38, × 110; Fig. 42, × 450; the rest, × 750.

cotyledon side of the embryo. A few endosperm cells may also be seen at the extreme chalazal end near the tip of the cotyledons in the mature seed. But these are generally in a degenerating state.

The cells of the endosperm contain numerous starch grains but some cases were noted where such grains were absent. In the mature seed the endosperm cells may show the presence of the normal nuclei but generally they are darkly stained and lobed showing signs of degeneration (Fig. 39). The tangential walls of the endosperm cells touching the embryo and testa become thickened (Fig. 39). This change is more pronounced in the cells at the micropylar end than in the cells situated towards the chalazal side where the amount of thickening is comparatively less. The radial walls of the endosperm cells also become slightly thickened.

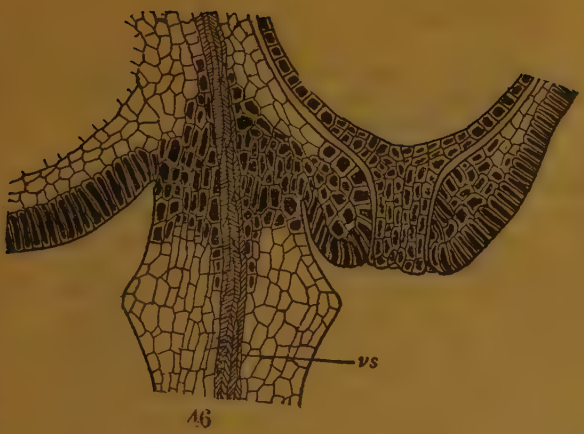
During the formation of endosperm the embryo-sac grows at the chalazal end, digesting its way through the nucellar cells and becomes curved. As the embryo-sac develops the cells in the chalazal region become enlarged, elongated and highly vacuolate before they are digested away by the advancing embryo-sac. Similarly the cells at the micropylar end also are crushed. Only the central part of the nucellus enclosed by the annular embryo persists in the mature seed. The cells of it contain numerous small starch grains. This is the principal storage tissue of the seed.

Testa

The ovules are bitegmic. The inner integument alone forms the micropyle as in most members of the Centrospermales (Figs. 46 and 55). At the time when tetrad of megaspores is formed, the outer integument towards the micropyle consists of two layers of cells which gradually increase up to four or five layers towards the chalazal end. During further development the number of layers in the outer integument continues to increase, especially on the chalazal side. In *Rivina humilis* the outer integument, during the early stages of embryo development, consists generally of three and sometimes of four layers in the micropylar part except at the tip where it is thicker, while six to eight layers are seen towards the chalazal end. In *Phytolacca dioica* at this stage there are generally three layers of cells at the micropylar end (the tip is thicker) and five to six layers in the chalazal part.

The inner integument in *Rivina humilis* consists mostly of two layers of cells except at the tip where it is thicker. In *Phytolacca dioica* the structure of the inner integument differs from that of *Rivina humilis* in that it has three or four layers of cells in the chalazal region.

Both the integuments take part in the formation of the testa which in the mature seed is composed of all the layers of the outer integument and the inner epidermis of the inner integument. The outer epidermis of the inner integument (and the layers derived from it towards the chalazal end in *Phytolacca dioica*) disappears during development of the testa (Fig. 44). At the tip, however, all the cells of inner integument persist in the mature seed. These are filled with brownish grains during the early stages of embryo development



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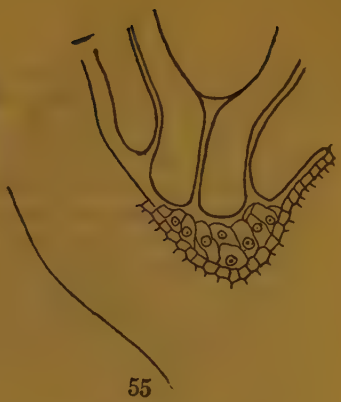

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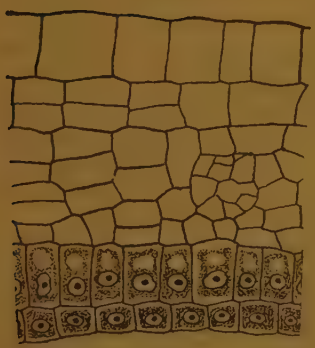

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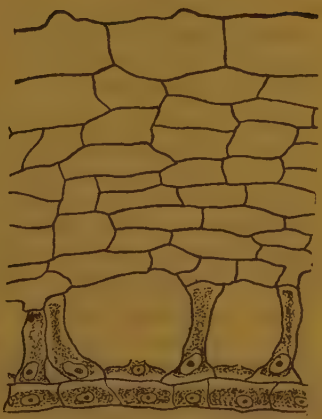

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FIG. 46. *Phytolacca dioica*. L.S. of the basal part of the ovule showing deposition of the grains in all the layers of the inner integument at the tip. Note the vascular strand (*vs*) and also the deposition of the grains in the upper part of the funiculus and outer integument. FIGS. 47–55. *Rivina humilis*.—Fig. 47. A seed. Note the absence of hairs around the hilum. Figs. 48–49. L.S. of a part of the ovary wall showing development of the hairs. Fig. 50. A mature fruit showing the presence of persistent style, perianth and filaments. Figs. 51–54. Apical parts of the hairs. Fig. 55. L.S. of a portion of the ovary near the micropyle showing obturator, ×450 except Fig. 46, ×175 and Figs. 47 and 50, × 17·5.

(Fig. 46). This was clearly noted in *Phytolacca dioica*. The ultimate fate of the corresponding cells in *Rivina humilis* could not be definitely ascertained, but they were seen to persist with a few yellow staining grains in them until the embryo becomes cordate.

As the development proceeds the cells of the outer epidermis of the outer integument become very conspicuous by their radial elongation (Figs. 40, 41 and 44). In these cells numerous grains, staining yellow with hæmatoxylin become deposited (Figs. 41, 43 and 44). The deposition of the grains commences as early as the megaspore mother cell stage in *Phytolacca dioica* and a little later in *Rivina humilis*. The grains appear first at the chalazal end. As the seed reaches maturity these outermost epidermal cells appear pillar-like in longitudinal section and are completely filled with the dark brown deposits forming a stony layer of the testa (Figs. 42 and 45). The radial walls of these cells also become somewhat thicker but the maximum increase in thickness is seen in the outer tangential walls as reported by Woodcock (1924) in *Phytolacca americana*.

The remaining layers of the outer integument on the inner side of the stony layer also turn brownish due to the deposition of the grains in them. The deposition in these layers starts much later than in the other layers (Figs. 41 and 45). Their cell walls also increase slightly in thickness. In the mature testa, studied only from *Rivina humilis*, these cells are tangentially elongated and are loaded with brownish contents. However, they appear somewhat crushed (Fig. 42).

The innermost layer of the testa is derived, as stated before, from the inner epidermis of the inner integument. The deposition of the grains in this layer starts from the micropylar end unlike that in the outermost stony layer. In *Rivina humilis* these cells in the mature testa also become tangentially elongated with contents of a slightly lighter shade than in the other layers. The most interesting point about them is their banded appearance when seen in longitudinal section (Fig. 42).

When the testa is being formed the deposition of the grains also takes place in the uppermost part of the funiculus and in the cells situated in the vicinity of the vascular strand that extends into the ovule (Fig. 46). The remains of the vascular strand along with the neighbouring cells loaded with these grains could be seen very clearly in the mature seed of *Rivina humilis* (Fig. 37).

Fruit

The structure of the fruit has been studied only in *Rivina humilis*. It is a small globular crimson red berry with a persistent style near its apex. The perianth lobes and the filaments of the stamens, mostly without anthers, also persist as greenish structures (Fig. 50).

The ovary wall consists of eight to ten layers of cells at the time of fertilization (Fig. 48). The number of these layers remains the same in the mature fruit. The cells at this time in almost all the layers show general increase in size. They are parenchymatous, juicy and show the presence of nuclei. The cells of the inner hypodermis, however, behave differently. These cells, as the fruit develops, become

radially elongated and form long hairy structures creating sufficient space in between the inner epidermis and the remaining layers of the pericarp (Fig. 49). The space thus formed becomes gradually filled with a coloured watery juice of the berry. The inner epidermis along with the hairs become adpressed to the testa as a result of which seeds appear copiously hairy.

The mature seed in *Rivina humilis* is biscuit-shaped and is very hairy (Fig. 47). When the hairs are removed they look black in colour. The hilum is present on one side in a shallow depression. Hairs are absent around it. The hairs are unicellular. They may be simple, or once or twice forked at the tip. The free ends may be blunt or pointed (Figs. 51–54).

Discussion

The preceding descriptions indicate that the embryo development in *Rivina humilis* and *Phytolacca dioica* conforms respectively to the Chenopodiad and Caryophyllad-type of Johansen (1950).

The origin, disposition, eventual destinations and the histogenic functions of the proembryonic cells in *Rivina humilis* agree in all essential respects with those of *Chenopodium bonus-henricus* (Souèges, 1920). The two cells, *ca* and *cb*, of the first cell generation behave as in *Chenopodium* (Souèges, 1920), the former giving rise to the two cotyledons, stem tip and lower part of the hypocotyl and the latter to the upper part of the hypocotyl and radicle, root cap and suspensor. The cell *cb* also forms the two histogenic layers, *i.e.*, the periblem and plerome of the embryo.

During second cell generation the proembryo in *Rivina* consists of a row of four cells formed by the transverse divisions of both the cells, *ca* and *cb*. There is a diversion of destinations at this stage. The cell (or tier) *l* forms the stem tip and two cotyledons. The upper tier *l'* gives rise to the lower part of the hypocotyl. The root tip, radicle and the upper part of the hypocotyl originate from the tier *m*. The cell *ci* engenders the root cap and suspensor.

In the third cell generation the embryo consists generally of eight cells disposed in five tiers due to the vertical divisions of the three apical tiers (*l*, *l'* and *m*) and a transverse division of the cell *ci* forming *n* and *n'*. The destinations of the three apical tiers remain the same as in the second cell generation. The cell *n* forms the root cap and a part of the suspensor. The remaining part of the suspensor is derived from *n'*.

It is thus clear that a considerable portion of the embryo proper in *Rivina humilis* is derived from the basal cell in very much the same manner as in *Chenopodium bonus-henricus* (Souèges, 1920). The only difference between these two species is in the behaviour of the tier *m*. In *Rivina*, it forms two tiers *ma* and *mb*, which are not organized in *Chenopodium* (Souèges, 1920) at such an early stage of embryo development as in *Rivina*.

No significant difference in the origin and structure of the suspensor is noted between these two plants. Thus except for the only

minor difference noted above the embryo development in *Rivina humilis* agrees very closely with that of *Chenopodium bonus-henricus* (Souèges, 1920) and conforms to the Chenopodiad-type of Johansen (1950).

The embryo development in *Phytolacca dioica* conforms in the main to the Caryophyllad-type of Johansen (1950) but there is a superposition of certain characters of the other types. The apical cell *ca* of the first cell generation engenders the stem tip, two cotyledons, hypocotyl, radicle and root cap. The basal cell *cb* forms the suspensor only.

In the second cell generation the cell *cb* divides transversely a little in advance of the terminal cell to produce *m* and *ci*. The apical cell *ca* also segments likewise and forms the cells *cc* and *cd*. Thus the filamentous form of the proembryo corresponds to that in the Chenopodiad-type (Johansen, 1950). The embryo proper originates from the cell *cc*. The cell *cd* develops into the root tip and root cap. The two cells *m* and *ci* derived from *cb* contribute to the suspensor. Thus the destination of the cell *cb* and its derivatives remain the same but the derivatives of *ca* (*cc* and *cd*) acquire new destinations during the second cell generation.

In the third and subsequent cell generations the tiers of the filamentous proembryo do not acquire new destinations. The tier *cc* divides first by two vertical walls forming a quadrant. Each cell of the quadrant divides transversely and an octant stage is reached. The four apical cells of the octant (*l*) give rise to the stem tip and two cotyledons. The other four cells (*l'*) form the hypocotyl and radicle. Thus the sequence of divisions in the tier *cc* and the histogenic role of the resultant octant are comparable to those in the Onagrad-type, or the Sedum variation of the Caryophyllad-type (Johansen, 1950). The tier *cd* forms a quadrant in which periclinal walls are cut off. All these cells divide to form two tiers. The lower tier forms the root tip and the upper one develops into the root cap. Thus the behaviour of the tier *cd* corresponds to that of the two tiers, *h* and *h'* in *Drosera rotundifolia* (Souèges, 1936 *b*). At this stage the divisions in the cells *m* and *ci* occur in irregular planes and a massive suspensor results.

Thus it is clear that the filamentous form of the proembryo conforms to the Chenopodiad-type (Johansen, 1950); the behaviour of the tier *cc* corresponds to that of the Onagrad-type or the Sedum variation of the Caryophyllad-type (Johansen, 1950) and the tier *cd* resembles the tiers, *h* and *h'* together, in *Drosera rotundifolia* (Souèges, 1936 *b*).

But on the whole the embryo development in *Phytolacca dioica* conforms to the Caryophyllad-type of Johansen (1950) as the embryo is produced almost entirely by the terminal cell of the filamentous proembryo while the cell *cb* and its derivatives take no part in its construction.

Further the embryo development in *Phytolacca* conforms to none of the variations under the Caryophyllad-type of Johansen (1950). The chief points of differences with the other variations are as follows:—

(1) There is no formation of the epiphysis in *Phytolacca* as in *Sagina, Vaccaria, Hypecoum* and *Corydalis*.

(2) There is an absence of swollen haustorial cells in the suspensor of *Phytolacca.*

(3) The suspensor is fairly massive and is formed entirely from the cell *cb.* The cell *ca* and its derivatives take no part in its construction as in the majority of the Caryophyllad-type (Johansen, 1950).

(4) With regard to the structure of the suspensor the case of *Phytolacca* is intermediate between *Saxifraga granulata* (Souèges, 1936 *a*) and *Fumaria officinalis* (Souéges, 1941) but it differs chiefly from the latter two cases in that no part of the suspensor is derived from the terminal cell (*ca*).

(5) As regards the behaviour of the cell *cb* forming the suspensor, *sensu stricto*, *phytolacca* agrees with *Drosera rotundifolia* (Souèges, 1936 *b*), but it differs from the latter in the structure of the suspensor and the disposition of the quadrants.

Therefore, a new variation designated as *Phytolacca* variation under the Caryophyllad-type of Johansen (1950) is proposed. The relevant part of the classification of the Caryophyllad-type by Johansen (1950) is reproduced below to indicate the place of *Phytolacca* variation. For details see (Johansen, (1950).

Mature embryo dicotyledonous:

1. Epiphysis present.
   Sagina variation.
   Vaccaria ,,
   Hypecoum ,,
   Corydalis ,,
2. Epiphysis absent
   (i) Basal cell (*cb*) never or very rarely divides again.
   Sedum variation.
   Medicago,
   (ii) Basal cell forms a suspensor.
   (*a*) Basal cell divides longitudinally to form two juxtaposed inflated cells.
   Myriophyllum variation.
   (*b*) Basal cell divides transversely: daughter cells are usually again segmented.
   Drosera variation.
   (*c*) Basal cell divides quite irregularly to form a comparatively large suspensor.
   I. Suspensor smaller than the embryo during all stages.
   Phytolacca variation.
   II. Suspensor bigger than the embryo almost till the latter is mature.
   Fumaria variation.

Summary

Fertilization and the development of embryo, endosperm and seed coat have been studied in *Rivina humilis* and *Phytolacca dioica*. The structure of the mature seed and the development of the fruit has been described in *Rivina humilis*.

Fertilization is porogamous. Double fertilization has been observed. During fertilization the nucellar cells situated directly below the micropyle become radially elongated. In *Rivina humilis* obturator is observed.

Occasionally, one or both the synergids persist during the early stages of embryo development inside the embryo sac. Likewise the pollen tubes also persist during the early stages of embryo development. The antipodals in *Rivina humilis* sometimes increase in number up to six and become laterally placed.

The embryo development in *Rivina humilis* conforms to the Chenopodiad-type.

The embryo development in *Phytolacca dioica* conforms to the Caryophyllad-type with a superposition of certain characters from the other fundamental types. A new variation designated as *Phytolacca* variation under the Caryophyllad-type for this species is therefore proposed.

The suspensor in both the species is quite massive.

The nuclear endosperm gradually becomes cellular throughout the embryo-sac. In the mature seed it is represented by a few layers forming a cap over the radicle. The central part of the nucellus persists as perisperm in the mature seed.

The testa is developed from all the layers of the outer integument and the inner epidermis of the inner integument. The cells of the outermost layer become radially elongated and palisade-like forming a stony layer of the seed coat.

The mature embryo in *Rivina humilis* is dicotyledonous and annular. The cotyledons are folded. The seeds are hairy. The hairs are derived from a portion of the ovary wall.

Acknowledgments

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STUDIES IN THE ORDER PARIETALES

IV. Vascular Anatomy of the Flower of Tamaricaceæ

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Introduction

The evidence available indicates that the order Parietales is not a phylogenetic taxon and that a realignment of the families into several orders is desirable (Lawrence, 1951). Work on the anatomy and morphology of the various families was started in this laboratory in 1939 with a view to find out how best this regrouping can be effected. The families that have been studied so far are Guttiferae (Puri, 1939 *b*), Passifloraceæ (Puri, 1947 and 1948), and Caricaceæ (Devi, 1952). Puri (1939 *a*), while dealing with the embryology of *Tamarix chinensis*, gave a brief account of the vascular anatomy of the flower of this species. Embryology of several other species of Tamaricaceæ also has been studied, but not their floral anatomy. Since material of several species of Tamaricaceæ was available in this laboratory the present work was undertaken at the suggestion of Dr. Puri.

Material and Methods

Altogether seven species of *Tamarix* and one of *Myricaria* have been worked out in the course of the present investigation. Material of *T. troupii* and *T. dioica* was collected from the banks of Hindan and Jumuna near Meerut, and that of *T. ericoides*, *T. troupii* and *T. articulata* was collected at my request by Mr. B. D. Tiagi from Ajmer and Mr. S. N. Chaturvedi from Agra. Three species of *Tamarix* (*T. pentandra*, *T. parviflora* and *T. odessana*) were collected by Dr. Puri from the Brooklyn Botanical Garden while he was in the U.S.A. in 1949. Herbarium material of *Myricaria germanica* was very kindly given to me by my colleague Mr. M. L. Banerji who collected it from Nepal in June 1948.

Dried floral buds of *Myricaria germanica* were first softened in 5% sodium hydroxide at 58° C. for about four hours and then thoroughly washed in running water for about six hours before they were passed through the ethyl alcohol grades. The buds so treated swelled almost to their normal form and gave good sections.

Serial microtome sections, both transverse and longitudinal, were cut 8–12 microns thick and stained with crystal violet and erythrosin. Herbarium material of *Myricaria*, however, stained better with safranin and fast green.

Observations

External Morphology.—The species of *Tamarix* examined are either shrubs (*T. dioica*, *T. troupii*) or trees (*T. articulata*). Their flowers are generally pedicellate or sub-sessile, bracteate (Lawrence's, 1951, description of them as ebracteate is apparently incorrect), regular, bisexual (diœcious in *T. dioica*) and pentamerous (tetramerous in *T. parviflora*) with tricarpellary gynæceum.

The sepals are quincuncial, slightly connate at the base and persistent. Petals are twisted, longer than the sepals and generally straight, but in *T. parviflora* they are strongly reflexed with their apical parts surrounding the sepals. The stamens are alternipetalous and equal in number to the petals except in *T. ericoides* where they are twice as many. The antesepalous stamens in this species have filaments longer than those of the antepetalous ones. Female flowers of *T. dioica* have staminodes with flattened filaments and pale sagittate anthers having sometimes poorly developed sporogenous tissue.

The ovary in all the species examined is unilocular with three or four parietal placentæ that project towards the centre. The ovarian cavity makes its appearance in the form of three loculi which soon merge into one. The ovary wall in *T. ericoides* is often found punctured by insects.

The outer epidermis of the ovary wall is characterised either by rectangular cells with flattened ends, *e.g.*, *T. pentandra*, *T. odessana*, *T. troupii*, *T. parviflora* and *T. chinensis*, or by elongate, palisade-like cells with pointed free ends, *e.g.*, *T. dioica*, *T. articulata* and *T. ericoides* (Figs. 22 and 23). The cells of the inner epidermis of the ovary wall in *T. odessana* and *T. troupii* are similar to those of the outer. These features are useful in distinguishing species and groups of species.

The fruit wall shows three equidistant longitudinal furrows, one outside every carpellary dorsal. These furrows represent the lines along which dehiscence of the mature fruit takes place.

Myricaria germanica Desv. resembles *Tamarix* species in most of the floral characters except in the presence of a staminal tube and absence of the disc at the base of the ovary. There are ten alternately long and short stamens. The gynæceum is tricarpellary with three sessile stigmas. The three placentæ in the basal region are adnate to one another (Fig. 40) but higher up they separate off and the ovary becomes unilocular (Figs. 41, 42). The placentæ in *Myricaria* are longer than those in *Tamarix*. The cells of the epidermis of the ovary wall are columnar with obtuse or pointed apices.

Vascular Anatomy.—Vascular ground plan in all the species of *Tamarix* examined follows the same pattern. It will, therefore, suffice to describe the condition in one species in detail and call attention to the differences in other species.

The peduncle of the inflorescence in *T. pentandra* shows a dissected siphonostele of about ten endarch, collateral bundles surrounded

FIGS. 1–10.

FIGS. 1–10. *T. pentandra* Pall. Fig. 1. Semidiagrammatic longitudinal section of the flower bud showing vascular supply of different floral parts. Figs. 2–4. Successive transverse sections through inflorescence axis showing differentiation of bract, pedicel and their vascular supply. Figs. 5–10. Serial cross-sections of a bud from pedicel upwards showing vascular supply of sepals, petals and stamens. *Br.*, Bract supply. *Pd.*, pedicel supply. Lines 13, 15, 18 indicate the levels at which the figures with the same numbers have been drawn.

by a ring of fibrous tissue. One bundle passes out from this ring corresponding to every flower (Fig. 2) and this soon splits into three, one outer and two inner (Fig. 3). The outer one supplies the bract and the two inner ones the pedicel of the axillant flower (Fig. 4). The single bundle that enters the bract soon divides into three and afterwards into many branches.

The remaining two bundles that are meant for the pedicel divide and redivide to form a cylinder of vascular tissue (Fig. 5). Five sepal traces more or less in a spiral succession (Fig. 6) pass out of this cylinder in the region of the receptacle. The first two traces are lateral, the third and fifth are anterior, while the fourth is posterior. The sepals separate off also in the same order. Every sepal trace divides into a mid-rib and two marginal bundles while still within the cortex of the receptacle (Fig. 7).

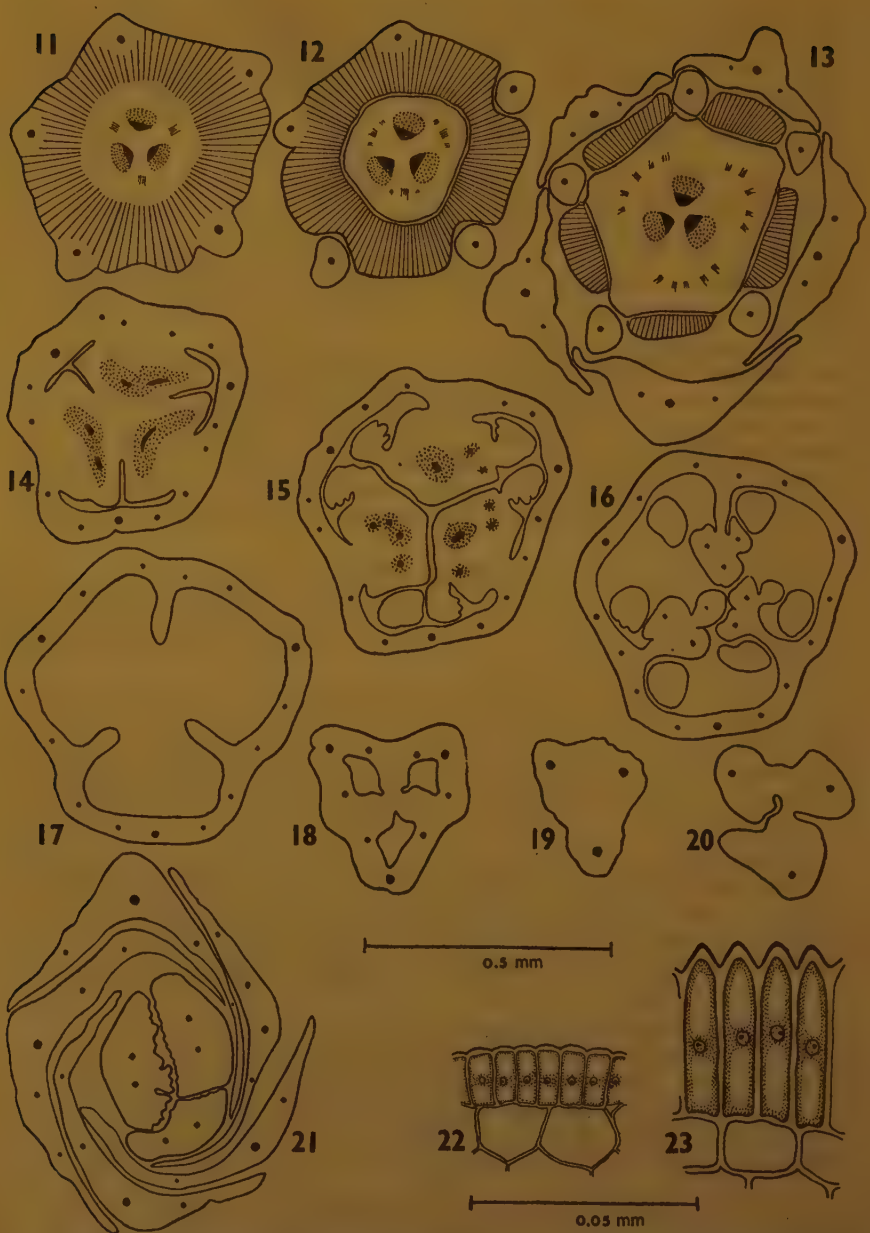
The sepal traces are immediately followed by the five petal traces which arise simultaneously (Figs. 7 and 8). Every petal receives a single trace that divides within the petal into a midrib and two or more laterals (Figs. 13, 21).

The gaps caused by the passing out of sepal and petal traces are soon bridged over and the vascular ring assumes a more or less five-sided appearance with the five ridges opposite the five sepals (Fig. 9). The traces for the stamens diverge simultaneously from the ridges and each enters one stamen (Figs. 9, 10). They remain undivided throughout their course.

After the departure of the staminal traces the tissue surrounding the base of the ovary is marked off by its capacity to stain deeply (Fig. 11). This represents the disc with the stamens inserted in its peripheral region. The disc breaks up after the separation of the stamens into five lobes, alternating with the five filaments (Figs. 12, 13).

The remaining vascular tissue soon organizes into a cylinder which gives off three prominent carpellary dorsals that continue right up to the stigma where they may undergo some forking (Figs. 10, 11 and 19–21). As the carpellary dorsals diverge out they give off on either side one secondary marginal which divides into 2–3 bundles, disappearing beyond the ovary region (Figs. 12–19).

The three bundles left in the centre after the departure of the carpellary dorsals become concentric (Figs. 10–14). They occur on alternate radii with the dorsals and are used up completely in furnishing traces to the numerous anatropous ovules that are borne on the basal region of the placentæ (Figs. 15, 16). Each placenta, in the



FIGS. 11–23. Figs. 11–21. Serial cross-sections of the flower bud of *T. pentandra* showing the course of bundles in the ovary. Figs. 22–23. Epidermal cells of the ovary wall of *T. pentandra* and *T. ericoides* respectively. Disc portion stippled.

upper region is represented by a septal outgrowth which projects more or less towards the centre (Fig. 17). As the top of the ovary is reached, these septal projections meet in the centre and thereby produce a trilocular condition, although only for a short distance (Fig. 18).

It will be seen that this account tallies in all essential respects with that of *T. chinensis* (Puri, 1939). The central plexus and the residual stelar tissue reported in the basal region of the ovary of *T. chinensis*, however, have not been observed in any of the other species studied here. The vascular anatomy of the flower of *Tamarix* (*T. parviflora* D C., *T. troupii* Hole, *T. articulata* Vahl., *T. odessana* Stev.,* and *T. dioica* Roxb.) also agrees with the account given above. There are some points, however, which deserve attention.

The ring of fibrous tissue that is present outside the vascular bundles of the peduncle in *T. pentandra* is not observed in *T. articulata*, *T. parviflora*, *T. odessana* and *T. ericoides*.

The single trace supplying a sepal in *Tamarix dioica* divides only after it has entered that organ. Sepals have thick fibrous tissue just within the inner epidermis while petals have this inside the outer epidermis. Besides, the fibrous tissue in the petals is more strongly developed at the tips than at the base. The pistillode of male flower exhibits three weak dorsals and small cavity. The disc with deeply cleft lobes is more prominent in the male flowers than in the female.

T. ericoides Rottl. differs from species described above in the vascular supply of the bract and in the presence of ten stamen traces. This appears to be a connecting link between *Tamarix* and *Myricaria*. It will, therefore, be worthwhile to deal with this species in some detail.

The inflorescence axis has a dissected siphonostele of 8–10 endarch, collateral bundles. Traces to the pedicel and its subtending bract are given off separately from the peduncle stele unlike the condition in other species where only a single bundle supplies both bract and pedicel. Every bract receives a single trace (Figs. 24, 25) that divides into three and finally into many (Figs. 26–28). The pedicel in the axil of the bract receives two traces that arise from the sides of the gap left after the passing out of the bract supply (Figs. 26, 27). These divide as they move outward and form a more or less complete ring at the level of separation of the pedicel (Fig. 28).

Vascular supply to the sepals and petals is similar to that of *T. pentandra*, but here the bundles divide several times so that each of the organs shows many bundles in a transverse section. The ten stamen traces that diverge out somewhat irregularly through the disc enter the filaments that separate off from the receptacle without any regularity. However, in some cases, antepetalous stamens separate off earlier and are shorter than the antesepalous ones (Figs. 29, 30). The separation of the stamens divides the disc into ten alternating short

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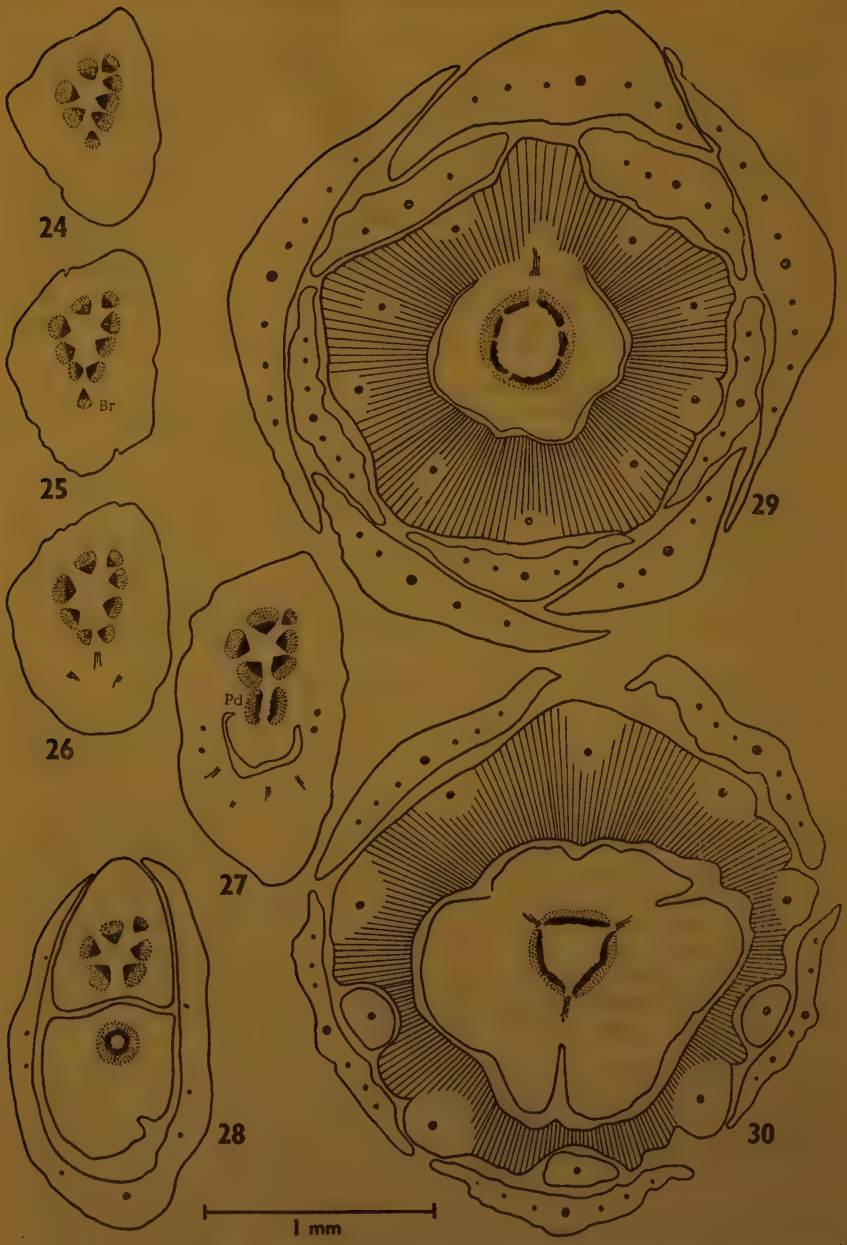
* Observations concerning this species are based on the slides prepared by Mr. A. C. Gupta.



FIGS. 24–30. Figs. 24–28. Successive transverse sections of the inflorescence axis of *T. ericoides* showing differentiation of bract, pedicel and their vascular supply. *Br.*, Bract supply. *Pd.*, Pedicel supply. Figs. 29–30. Cross-sections of the flower bud of *T. ericoides*. Fig. 29. Shows that antepetalous stamen traces have reached the periphery of the disc. Fig. 30. Filaments of three antepetalous stamens are already marked off from the disc while the traces of the antesepalous ones have reached only the periphery of the disc.

lobes, which are non-vascular. The vascular supply of the gynæceum is similar to that of *T. pentandra.*

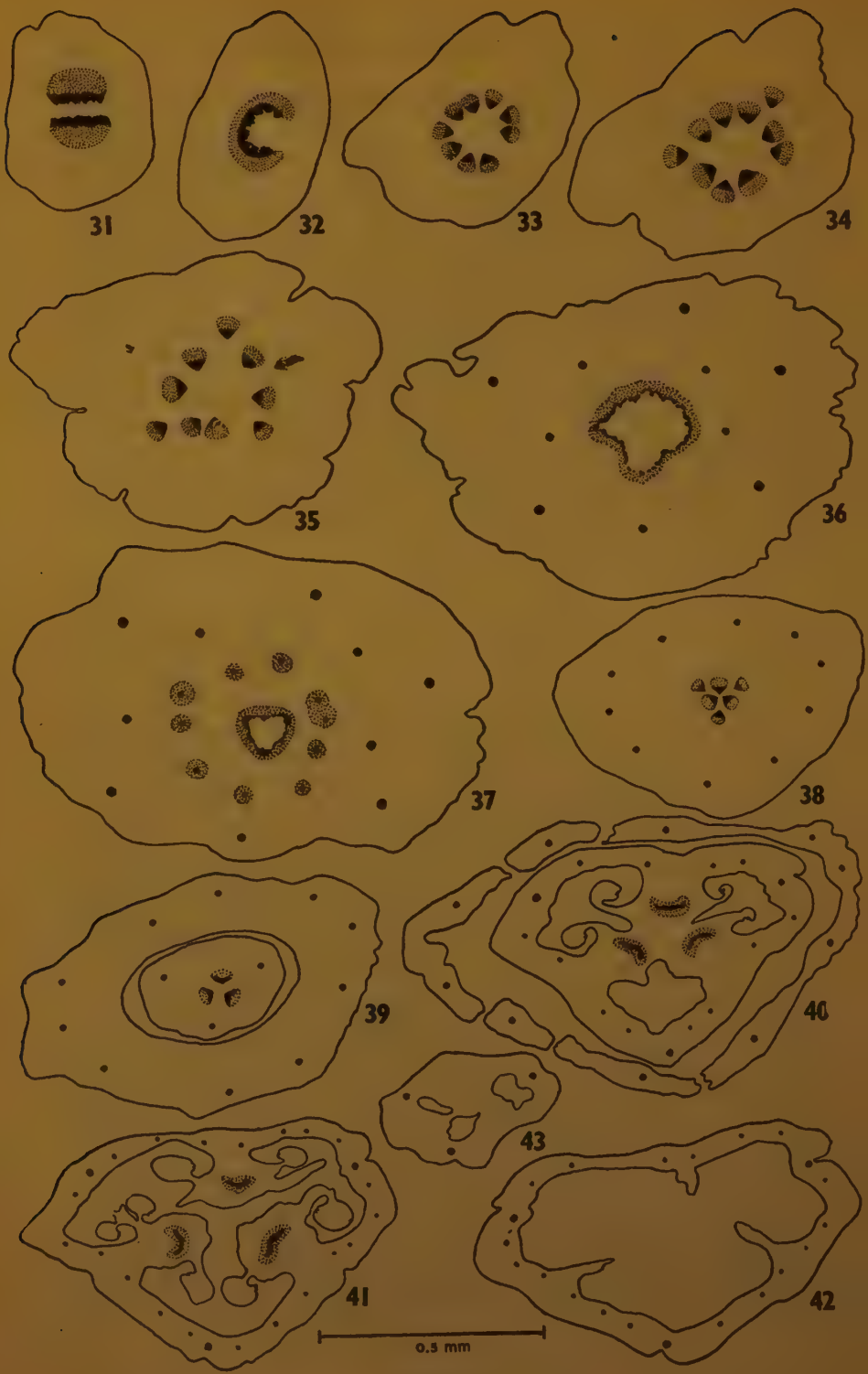
In *Myricaria germanica* Desv. the vascular supply of the bract and pedicel is exactly similar to that of *T. ericoides.* The pedicel at its base contains two bundles (Fig. 31) that divide and redivide to form a horseshoe-shaped structure (Fig. 32). A little higher up it breaks up into 8–10 collateral endarch bundles arranged in a ring (Fig. 33). The five sepal traces which are given out in spiral succession traverse undivided within the cortex of the receptacle (Figs. 34–36). Petals also receive one trace each and all the petal traces arise almost simultaneously (Fig. 36). All the ten stamen traces leave the stele practically at the same level (Figs. 37, 38). They diverge out a little and enter a parenchymatous cylinder which in the meantime separates off from the ovary as well as from the floral tube (Fig. 39). This cylinder is formed apparently by the fusion of staminal filaments for about half their length. In the upper region, the tube splits up into ten stamens and no lobes of the type met with in *Tamarix* are observed in-between the stamens (Fig. 40). The course of vascular bundles in the ovary (Figs. 41–43) resembles that of *Tamarix.*

Discussion

The study of the floral anatomy of seven species of *Tamarix* and one species of *Myricaria* reveals a remarkable uniformity in the general plan of the flower and the vascular supply of the different floral parts.

The bract trace in *T. ericoides* and *Myricaria germanica* first comes out of the stele of the peduncle leaving a gap while the two bundles on the sides of the gap form pedicel traces. In other species studied a single bundle comes out of the stele of the peduncle which divides into an outer bract trace and two inner pedicel traces. This is indeed a valuable observation in so far as it shows that the condition in *T. ericoides* and *Myricaria* is more primitive and that in other species it has been complicated by adnation and cohesion of traces. Cohesion of traces is also met with in *T. dioica* and *Myricaria* where sepal marginals are fused with their dorsals throughout the cortex of the receptacle. It is interesting to see that *Myricaria* which is primitive in many features appears to be more advanced in this particular respect.

The Andrœcium.—Niedenzu (1925) describes the andrœcium of *Tamarix* as both obdiplostemonous and haplostemonous while that of *Myricaria* as more or less distinctly obdiplostemonous. Warming and Potter (1932) also considered the andrœcium in two whorls, the inner one being absent in *Tamarix.* Vascular anatomy does not seem to offer a satisfactory confirmation of these inferences, for in both *T. ericoides* and *M. germanica* where ten stamens occur all staminal traces arise practically at the same level. It is seen, however, that in some cases, the staminal traces in the disc of *T. ericoides* arrange themselves in two more or less distinct rings, the antepetalous traces forming the outer whorl (Fig. 29). Further, in some cases of *T. ericoides* most of the antepetalous stamens separate off at a somewhat lower level than the rest (Fig. 30). Besides, the antepetalous stamens are



FIGS. 31–43

FIGS. 31–43. Figs. 31–43. Serial cross-sections of a bud of *Myricaria germanica* from pedicel upwards. Fig. 40. Shows the splitting up of the staminal tube into filaments. Carpellary dorsals and ventrals on different radii are also clear.

shorter than the antesepalous ones. All the staminal traces in *M. germanica* leave the central stele practically at the same level and occur in the same ring within the staminal tube. Higher up this staminal tube splits up into ten alternately long and short stamens which form a single whorl. Thus, whatever anatomical evidence of obdiplostemony was observed in *T. ericoides* does not exist in *Myricaria*.

The Disc.—A prominent disc consisting of deep staining tissue occurs surrounding the base of the ovary in all species of *Tamarix*, but it is absent in *Myricaria*. Higher up when the stamens separate off the disc splits up into five (*T. pentandra*) or ten lobes (*T. articulata*) alternating with the stamens. These small parenchymatous lobes are without any vascular tissue. They have been described as glands (Hooker, 1875) and stipular teeth (Warming and Potter, 1932). The fact that they lack any vascular tissue within them rules out the possibility of their being interpreted as staminodal in nature. They are perhaps best described as being the fusion products of adjacent stipules of adjoining stamens, for it is well known that stipules can be without any vascular tissue (Glück, 1919). It appears logical, therefore, to conclude that the disc is staminal in nature being formed by the staminal bases and their stipules.

The Gynæceum.—The vascular supply of the ovary is uniform in all the species of the two genera examined. The mid-rib bundles in *T. ericoides* and *T. pentandra* often divide into two each as the stigma is approached. Such dichotomies have been very extensively commented upon in attempts to apply telomic conception to angiosperm carpels (Thomas, 1934; Hanf, 1935; Hunt, 1937, etc.).

Another feature, which deserves some attention here, is the multilocular condition of the ovary at its base and near its top. Such a condition, although easily understandable on the basis of the classical interpretation of the carpel cannot be explained if we follow rigidly the classification of the carpels put forward by Troll (1928). For instance, the *Tamarix* gynæceum according to his classification is essentially paracarpous, the extreme base which is multilocular being "syncarpous" (*sensus restricto*). How paracarpous carpels can become multilocular in the upper region as they do in *Tamarix* is not easily understandable. Arber (1942) and Puri (1950) have also drawn attention to such features. It, therefore, seems logical to conclude that Troll's distinction between syncarpous and paracarpous carpels cannot be maintained.

The Placentation.—The placentation in *Tamarix* has been variously described as basal (Hutchinson, 1926; Warming and Potter, 1932: Joshi and Kajale, 1936; Sharma, 1939; and Gundersen, 1950), reduced to basal (Lawrence, 1951); and parietal on its way to basal (Puri, 1939). In a recent comprehensive review Puri (1952) described and redefined the types of placentation. He suggested that the term basal placentation should be applied to the conditions where a single ovule arises or

appears to arise from the bottom of the ovary. Since there are many ovules and the placentæ also continue for quite a distance beyond the base of the ovary the condition in *Tamarix* cannot be described as basal. Following Puri's classification the placentation in all the species studied here, is parietal. Even in the basal region where the gynæceum is generally multilocular (cf. *Myricaria*) the anatomy is that of parietal placentation, that is the placental strands are formed by the fusion of ventrals belonging to different carpels and they occur on radii different from those of the dorsals. Sharma (1939) who reports trilocular ovary in *T. ericoides* evidently observed transverse sections through the basal region of the ovary only.

Apparently the placentæ in the Tamaricaceæ are undergoing reduction. In the less specialized genus *Myricaria* they are fairly extensive and bear a larger number of ovules, but in *Tamarix* species they have become reduced considerably in their vertical extent. It is quite likely that in course of time they may undergo further reduction and the number of ovules may also get reduced to one per ovary. Even then, the placentation may not become typically basal. It will be lateral or sub-basal as in Gramineæ or Piperaceæ (Murty, 1952). The author concurs with Puri (1952) that typical basal placentation is obtained from axile or free central placentation. Therefore, to describe the placentation as basal in Tamaricaceæ is not quite correct.

Phylogenetic Considerations.—Niedenzu (1925) divides the family into Reaumurieæ containing the genera *Reaumuria* and *Hololachne*; and Tamariceæ with *Tamarix* and *Myricaria*. The section Reaumurieæ, especially the genus *Reaumuria*, with generally solitary flowers, 10-many stamens, 5-carpellary ovary, and many ovules, appears to be the least specialized of all. *Tamarix*, one of the two genera of Tamariceæ, is characterized by five stamens, considerably reduced placentæ with comparatively fewer ovules and bract supply adnate to the pedicel supply. *Myricaria*, on the other hand, has 10 stamens, elongated placentæ, independent bract supply and no disc. In view of these considerations *Myricaria* appears to be less specialized than *Tamarix*. *Tamarix ericoides* with 10 stamens and independent bract supply, however, forms a connecting link between the two genera.

Diœcism met with in *Tamarix dioica* also appears to be an advanced character for it does not occur in any of the less specialized genera.

Niedenzu (1925) further divides *Tamarix* into two sections, Sessiles and Amplexicaules. Five of the eight species examined here, *e.g.*, *T. troupii*, *T. pentandra*, *T. odessana*, *T. chinensis* and *T. parviflora*, fall under the first section and the other three, *e.g.*, *T. articulata*, *T. ericoides*, and *T. dioica* under the second. It is interesting to note that these two groups can be distinguished from one another on the basis of characters of the epidermal cells of the ovary wall. The Sessiles species, for instance, have small rectangular cells whereas those of Amplexicaules have long columnar cells with pointed apices. It will indeed be worthwhile to determine if this distinction holds good for all the species belonging to the two sections.

Niedenzu (1925) and Warming and Potter (1932) have called attention to the relationships of Tamaricaceæ and Salicaceæ on account of resemblances in ovary, placentation, ovule, fruit and seed characters. Pax, however, opined that the two families have nothing significant in common. Recent work of Fisher (1928), Maheshwari and Roy (1951), Nagraj, (1952) etc., have lent further support to this opinion. They are very different in characters of leaf, flower, embryo-sac and seed and whatever similarities there are, are just superficial. For instance, seeds are comose in both the families but the hairs in Tamaricaceæ develop from the epidermal cells of the chalazal end, whereas in *Populus* they are formed by the epidermal cells of the placentæ (Nagraj, 1952).

Summary

External morphology and vascular anatomy of the flower of seven species of *Tamarix* and one species of *Myricaria* have been described.

The bract and pedicel traces in some species arise quite independently, but in others, apparently more specialized forms, the two are adnate for some distance. Sepals receive three traces each whereas petals and stamens receive only one trace each.

Floral anatomy does not lend any support for the obdiplostemonous condition reported in *Tamarix* and *Myricaria*. The disc is believed to represent the fused bases of the stamens and their stipules.

The occurrence of trilocular condition in the upper and lower parts of the ovary is interpreted as evidence against Troll's classification of carpels into "paracarpous" and "syncarpous".

Placentation is described to be parietal. It is suggested that *T. ericoides* forms a connecting link between *Tamarix* and the less specialized *Myricaria*. The diagnostic importance of the peculiar epidermal cells of the ovary wall in the two sections of the genus *Tamarix* is emphasised and the view that Tamaricaceæ is not related to Salicaceæ is supported.

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VERNALISATION IN SOME VARIETIES OF INDIAN WHEAT

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Introduction

The results obtained by different workers on low temperature vernalisation on Indian wheats are at variance with each other. Kar (1940, 1942–43, 1946) could not obtain any earliness in ear emergence by pre-sowing chilling treatment of 7 varieties of wheat, while Sen and Chakravarti (1945) observed a significant earliness of 7–27 days in a set of 9 varieties. Working at New Delhi and Almorah, Sen, Chakravarti, Pal and Murty (1946) reported an earliness of 10–29 days in another set of 13 varieties. Pal and Murty (1941), however, noted very slight acceleration in their experiments with Indian wheats sown simultaneously at Delhi and Simla.

In the present investigation the vernalisation response of the two strains of mutant wheat, R-1 and R-9, considered to be the best (Pugh, 1945) among the 11 mutants obtained by Ranjan (1940) through X-ray irradiation of N.P. 52 has been studied.

Materials and Methods

Seeds of nearly uniform size, after being sterilised in corrosive sublimate solution (1 : 200) for 15 minutes, were soaked in water at the rate of 50% per fresh weight of the grains for 24 hours at room temperature to initiate embryo activity. The swollen seeds were placed in a refrigerator at 3–5° C. in lots for varying periods of 8, 13, 17 and 21 days. A few drops of water was added at intervals to keep the seeds in a moist condition. The covers of the Petri dishes were slightly lifted for aerating the seeds for some time each day. A set of untreated seeds was previously soaked in water for 24 hours to bring them to the same stage of development as the treated ones. Both treated and untreated seeds were sown in separate earthenware pots (11″×10″) containing a mixture of well pulverized garden loamy soil and cow dung (100 gm. per pot) in 6 replicates with 4 seeds in each pot. The treatments were adjusted in such a manner that the sowing was done on the same day. The pots were kept in an open space in the Botanical Garden under identical conditions of light and temperature. Later on when the seedlings attained the age of 30 days, ammonium sulphate at the rate of 1 gm. was added to each pot of both the control and treated sets. Care was exercised to maintain uniform soil moisture throughout the test by frequent application of water.

Experimental Results

Effect on the vegetative stage.—Two clear after-effects of the pre-sowing low temperature treatment for different periods were noticed.

(1) In all the treated sets, the seeds sprouted 2–3 days earlier than the controls.

(2) During the first 20–25 days growth, the young seedlings from the treated seeds were markedly different from those of the untreated control seeds in having stouter shoots with dark green colour of the leaves. This difference, however, gradually disappeared with increasing age of the seedlings.

The time of appearance of the tillers on the average was almost the same in both the treated and control sets. In Table I is given the mean number of tillers on the 35, 50, 65 and 80 days from the date of planting due to each treatment against the control.

Table I

Number of Tillers per Plant (Average of 24 Plants)

Period of Vernalization	8 days	13 days	17 days	21 days	Control
		Age at time of 1st count: 35 days			
Varieties—					
R—1 ..	4·45	3·46	3·91	3·00	4·54
R—9 ..	5·12	4·66	3·79	4·25	5·25
Mean ..	4·78	4·06	3·80	3·62	4·89
		Age at time of 2nd count: 50 days			
R—1 ..	7·04	5·50	5·66	4·12	7·20
R—9	6·75	7·04	5·37	5·08	7·20
Mean ..	6·89	6·27	5·51	4·60	7·20
		Age at time of 3rd count: 65 days			
R—1 ..	7·45	6·29	6·16	5·00	6·87
R—9 ..	6·25	5·79	4·91	3·66	6·04
Mean ..	6·85	6·04	5·53	4·33	6·45
		Age at time of 4th count: 80 days			
R—1 ..	3·50	3·37	3·37	3·29	3·45
R—9 ..	3·00	2·83	2·54	2·70	2·95
Mean ..	3·25	3·10	2·95	2·99	3·20

Though tiller emergence occurred in about the same time in all the treatments, yet the cold treated plants had a lesser number of tillers

throughout the major portion of the life-history. Due to drying out of some of the tillers, the number decreased in later stages.

It is seen (Tabe II) that the height of the plants from seeds vernalised for 13–21 days was considerably greater at every stage over that in the control.

TABLE II

Height per Plant in cm. (Average of 24 Plants)

Treatments	Vernalized for 8 days	Vernalized for 13 days	Vernalized for 17 days	Vernalized for 21 days	Control
	Age at time of recording height: 35 days				
Varieties—					
R—1 ..	26·78	32·64	31·25	35·37	25·79
R—9 ..	28·60	32·97	29·71	34·56	27·31
Mean ..	27·69	32·80	30·48	34·96	26·55
	Age at time of recording height: 50 days				
R—1 ..	48·50	52·53	59·27	58·12	49·20
R—9 ..	54·37	57·08	55·95	61·16	54·39
Mean ..	51·43	54·80	57·61	59·64	51·79
	Age at time of recording height: 65 days				
R—1 ..	65·81	71·35	69·06	70·08	61·25
R—9 ..	67·08	66·97	63·75	66·12	64·45
Mean ..	66·44	69·16	66·40	68·10	62·85

Effect on reproductive stage.—The results presented in Table III show clearly that in the control plants the ear emergence began with a very slow rate, the initial value on March 2 being 12·0% and 17·1% in the two varieties R-1 and R-9. Compared to this, the output of ear bearing tillers on that day under 21 days pre-chilling conditions was 27·5% and 53·4% respectively. This increased output of ear bearing tillers was maintained almost throughout the life period of the plant. Therefore, pre-chilling treatments for different durations not only enhanced the development of reproductive stage, but also brought forward the maximum number of ear bearing tillers in minimum of time.

Time of heading—The first emergence of the ear out of the leaf-sheath was taken to indicate the date of first bloom. The data with statistical analysis are presented in Table IV. The photographs of the treated and control plants are given in Plate (Figs. 1 and 2).

A study of Table IV shows that pre-sowing low temperature treatment produced slight earliness in ear emergence in both the strains

TABLE III

After-Effects of Pre-Sowing Chilling Treatment for Different Periods on the Percentage of Ear Bearing Tillers

	2-3-49		8-3-49		14-3-49		21-3-49	
	R-1	R-9	R-1	R-9	R-1	R-9	R-1	R-9
Vernalized for 8 days	14·5	19·3	44·7	65·9	78·0	95·9	86·9	98·6
do for 13 days	14·5	23·7	59·0	66·3	88·0	88·8	93·8	94·1
do for 17 days	18·9	27·1	47·9	70·3	88·8	90·6	88·8	96·7
do for 21 days	27·5	53·4	58·2	84·4	86·4	98·4	87·5	100·0
Control ..	12·0	17·1	29·7	52·3	72·9	92·1	84·2	95·9

TABLE IV

Time from Planting till Ear Emergence of the Main Shoot

Planting date, December 27 1948. + Indicates Earliness; —Indicates Delaying effect.

Treatments	No. of days from sowing to ear emergence of main shoot			Difference from control in days		
	R-1	R-9	Mean	R-1	R-9	Mean
Vernalized for 8 days ..	61·16	58·54	59·85	+1·13	+1·04	+1·08
do for 13 days ..	60·95	57·50	59·22	+1·34	+2·08	+1·71
do for 17 days ..	59·75	56·12	57·93	+2·54	+3·46	+3·90
do for 21 days ..	59·08	54·83	56·95	+3·21	+4·75	+3·98
Control ..	62·29	59·58	60·93			

S. E. of a treatment mean = 0·37; C. D. at 5% of a treatment mean = 1·05.

S. E. of an individual mean = 0·52; C. D. at 5% for comparing two individual means = 1·48.

of mutant wheat under observation. Considering the average effects of the treatments it is observed that at 5% level the sets vernalised for 17 and 21 days flowered significantly earlier than the controls. There is, however, no significant difference between the controls and the 8-day set on the one hand and the 8-day and the 13-day sets on the other. At the 1% level, the 17- and 21-day vernalised sets flower significantly earlier than the controls and the 8-day sets, while the difference between the controls, 8-day and 13-day sets and that between the 13- and 17-day sets are non-significant. This acceleration in ear emergence under the pre-chilling treatments for 17 and 21 days although statistically

significant is too small to be of practical importance in the climatic conditions of India.

Grain yield.—The grain yield of the individual plants with statistical analysis is presented in Table V. There was no significant increase in yield in any of the treated sets from the controls.

TABLE V

Grain Yield per Plant in gm.

Varieties ..	R-1	R-9	Mean	Percentage
Treatments Vernalised for 8 days	3·09	2·82	2·96	115·6
do for 13 days ..	2·77	2·62	2·69	105·1
do for 17 days ..	3·18	2·58	2·88	112·5
do for 21 days ..	2·68	2·76	2·72	106·2
Control ..	2·50	2·62	2·56	100·0

S. E. of a treatment mean = 0·15; C. D. at 5% of a treatment mean = 0·42.

S. E. of an individual mean = 0·21; C. D. at 5% for comparing two individual means = 0·59.

TABLE VI

Components of Yield (Average of 24 Plants)

Treatments	Vernalized for 8 days	Vernalized for 13 days	Vernalized for 17 days	Vernalized for 21 days	Control	S.E. & C.D. at 5% of a treatment mean	S.E. & C.D. at 5% for comparing two individual means
			No. of ears				
Varieties—							
R-1 ..	3·12	3·16	2·95	2·91	3·00		
R-9 ..	2·95	2·66	2·75	2·70	2·91	0·14	0·20
Mean ..	3·04	2·91	2·85	2·81	2·95	0·41	0·58
			No. of grains per ear				
R-1 ..	28·08	24·68	28·91	25·10	25·03		
R-9 ..	27·70	29·91	28·00	29·01	28·08	0·87	1·23
Mean ..	27·89	27·30	28·37	27·05	26·55	2·47	3·50
			One thousand grain weight in gm.				
R-1 ..	35·51	35·35	38·06	36·78	33·10		
R-9 ..	34·51	33·41	33·63	35·16	31·96	0·56	0·79
Mean ..	35·01	34·38	35·85	35·97	32·53	1·59	2·25

The components of yield such as number of ears, number of grains per ear and one thousand grain weight are given in Table VI. So far as the number of ears per plant and number of grains per ear are concerned there was no significant difference between the treated and control plants. The weight of grains obtained from treated plants, however, showed slight increase from that of the control set.

Discussion

Conflicting opinions prevail among the Indian workers with regard to the importance and necessity of pre-sowing cold temperature in the life-cycle of Indian wheats for successful production of flowers and fruits. The investigations carried out at New Delhi (Pal and Murty, 1941) and at Almorah (Sen and Chakravarti, 1945) have shown beyond doubt that the varieties of English winter wheat Holdfast, Little Joss, Yeoman, Juliana and Yorkwin have all given significant vernalisation response. Further, in these English winter wheat varieties, long days following chilling greatly increase the earliness. In the absence of chilling, long days have no effect. The picture is different for Indian varieties of wheat. In the two strains of mutant wheat long days alone (Misra, 1949, 1953) have brought about a significantly earlier ear emergence. The earliness is perceptible even with 8 long days. Similar effect of long days alone on other varieties of Indian wheats N.P. 52, N.P. 4, N.P. 165, N.P. 114 have clearly established the importance of post-sowing day lengths and high temperature on the development of the reproductive phase.

Acceleration of flowering in winter wheats of temperate zones by low temperature treatment has been accomplished by several workers (*cf.* Whyte, 1948, 1949). In a few varieties of Indian wheat an acceleration of 10–29 days by pre-chilling treatments has been noted (Sen and Chakravarti, 1945; Sen, Chakravarti, Pal and Murty, 1946). In the present investigation with two wheat mutants an acceleration of only 1–5 days was observed. This difference though statistically significant is too small to be of any agricultural importance (Misra, 1954). Kar (1940, 1942–43) could not obtain any acceleration from pre-chilled seeds when they are grown under natural days. This has led Kar (1946) to the generalization that to induce significant earliness from pre-chilled seeds of cultivated Indian wheat, it is essential that seedlings be subjected to a certain number of long days. In view of the findings of Sen, Charkavarti, Pal and Murty (1946) Kar's generalization seems to be untenable. The response of Indian varieties to vernalisation treatment is, therefore, varietal, striking variations being observed from variety to variety. Further, the method of application and the duration of cold treatment also differs in the experiments of different workers. For instance, Kar used a period of 7–30 days for vernalising, while Sen and Chakravarti vernalised the seeds for 63 days and Sen, Chakravarti, Pal and Murty for 7 and 28 days. In the present investigation a period of 8–21 days was used. The difference in the vernalisation response found between different workers might partly be due to this difference in the duration of cold treatment.

It has been proved beyond doubt that in the varieties of wheat which do not show any marked earliness in ear emergence there is need of a minimum low temperature for their normal growth and development. When the Indian wheats were grown in summer at Delhi (Pal and Murty, 1941) they did not set any seed. Wheat being a winter crop, its low temperature requirement under Indian conditions is met by sowing in winter. Pre-sowing cold treatment favours an increase in green pigment in the young seedlings (Kar, 1937; 1942–43). In addition to these a greater percentage of tillers came to bear ears at the outset in comparison with the control (Table III). Thus, though the pre-sowing low temperature brought about a small amount of earliness in these two strains of wheat, its after-effects are clearly perceptible. The response to vernalisation in temperate cereals is also partly connected with the type of thermophase and the degree to which this phase has been completed prior to seed maturity (Gregory and Purvis, 1936 and 1938). It has been found that in spring wheat, for instance, the ripening period lasts longer than it does in winter wheat and that considerable development takes place in the embryo, during the fruit formation and seed ripening in cereals. Under the tropical conditions wheat matures in comparatively longer daylight periods and warmer temperatures. Further work on this interesting point will throw new light on this differential response to vernalisation treatments of temperate and tropical wheats and the correlation of the pre-sowing thermal treatments with the after-sowing environmental conditions subject to variation by the experimental methods and the place of test.

It can be stated that a vernalisation phase as is understood for the temperate cereals does not exist in case of the majority of the cultivated Indian wheats. In contrast to severe climatic conditions in temperate zones in most parts of India the wheat plants do not receive extreme cold or frost. Rather, they get warmer climatic conditions and short day lengths. Although a slight dose of cold treatment is essential for the proper growth and reproduction for all the cultivated varieties of Indian wheat, for the majority of them the significance of vernalisation phase as far as its effect on enhancement of reproduction is concerned has been lost.

Summary

Two varieties of Indian wheat R-1 and R-9 were vernalised at 3-5° C. for varying periods of 8, 13, 17 and 21 days. In the young stage, the treated seedlings were markedly different from those of the untreated ones in having stouter shoots with dark green colour of the leaves. The treated plants had less number of tillers but attained greater height in comparison with the control. The plants obtained from seeds vernalised for 17 and 21 days flowered significantly earlier than the control, but the earliness is too small to be of any practical importance. The treatments did not bring about any change in the grain yield. The majority of the cultivated Indian wheats unlike their allied relatives of the temperate zone do not appear to have any special requirement of pre-sowing cold temperature.

Acknowledgments

Grateful thanks are due to Prof. Shri Ranjan for his guidance and helpful criticism throughout the progress of the work and the facilities provided at the Botanical Laboratory, Allahabad University, for carrying out this investigation. I am also thankful to Mr. T. P. Abraham, former Statistician, Central Rice Research Institute, Cuttack, for his advice in the statistical analysis of the experimental data. My thanks are due to Prof. B. Samantarai, Ravenshaw College, Cuttack, for kindly going through the manuscript and suggestions for its improvements.

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Explanation of Plate

Fig. 1. Earliness in heading in the plants of variety R–1 wheat obtained from low temperature vernalised seeds in comparison with the controls which received no treatment. Seed sown December 27, 1948. Photographed February 23, 1949.

Fig. 2. Earliness in heading in the plants of variety R–9 wheat obtained from low temperature vernalised seeds in comparison with the controls which received no treatment. Seed sown December 27, 1948. Photographed February 23, 1949.


VERNALIZED FOR 21 DAYS
FOR 17 DAYS
FOR 13 DAYS
FOR 8 DAYS
CONTROL

FIG. 1


CONTROL

FIG. 2

EMBRYOLOGICAL STUDIES IN THE LEGUMINOSÆ

VII. Endosperm and Embryo Development in *Neptunia triquetra* Benth. and *Prosopis spicigera* Linn.

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(Received for publication on March 15, 1954)

The present paper embodies a detailed study of the development of the embryo and a few more observations on the endosperm in *Neptunia triquetra* and *Prosopis spicigera* of the Mimosaceæ. The author has already recorded some observations on the subject while giving an account of the embryology of the above plants in the fourth (1952) and the fifth (1953) papers respectively of the above series.

Material and Methods

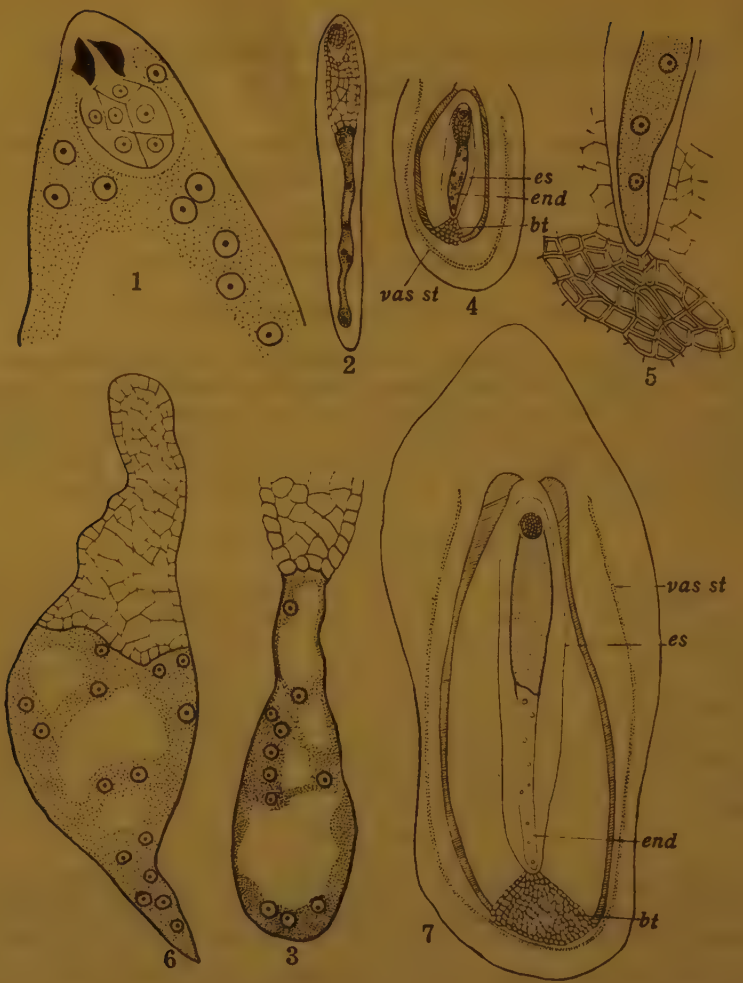
Material of *Neptunia triquetra* was collected from the compound of the College of Science, Nagpur and the Telenkheri area, Nagpur and that of *Prosopis spicigera* from the Mahal area, Nagpur. It was fixed in formalin-acetic-alcohol and Randolph's modification of Navaschin's fluid. Sections cut at thickness of 10–15 μ were stained with iron-alum hæmatoxylin, Harris' hæmatoxylin and Ehrlich's hæmatoxylin. Eosin was used as a counter-stain in some cases. Specimens of the dissected endosperm were stained with Zirkle's acetocarmine.

Endosperm

The endosperm follows the Nuclear type of development. Its formation has already been described in detail by the author in *Prosopis spicigera* (1953). More or less similar is the case in *Neptunia triquetra*.

The chalazal part of the endosperm is formed into a tube with free nuclei (Figs. 2, 3, 6). This tubular process comes in deep contact with the surrounding chalazal tissue of the nucellus. It remains free nuclear even as late as the appearance of cotyledons in the embryo and is seen clearly in whole mounts (Figs. 3, 6) dissected out from young seeds.

As the embryo-sac elongates in the chalazal direction of the developing seed, it brings about disorganisation of the surrounding nucellar tissue. This activity of the antipodal end of the sac is brought to an end by the formation of a barrier tissue by the nucellar cells in the chalazal region (Figs. 4, 5, 7). These cells become thick-walled and filled with a brown pigment probably tannin. The formation of the barrier tissue has been reported by Rau (1951 and 1953) in *Glycine*,



FIGS. 1–7.

Figs. 1–5. *Neptunia triquetra*.—Fig. 1. Micropylar part of embryo-sac showing persistent degenerated synergids, 6-celled proembryo and endosperm nuclei. Fig. 2. Embryo-sac showing massive proembryo surrounded by cellular part of endosperm, and narrow tube with free nuclei formed by chalazal part of endosperm. Fig. 3. Whole mount of endosperm showing chalazal tubular process with free nuclei. Fig. 4. L. S. of part of developing seed. *bt*, barrier tissue ; *end*, endosperm; *es*, embryo-sac; *vas st*, vascular strand. Fig. 5. Barrier tissue below antipodal end of embryo-sac. Figs. 6–7. *Prosopis spicigera*.—Fig. 6. Whole mount of endosperm showing micropylar cellular part and chalazal tubular process with free nuclei (embryo not shown). Fig. 7. L. S. of part of developing seed showing formation of barrier tissue. Legends same as for Fig. 4. Fig. 1, × 500 ; Figs. 2, 3, 5, 7, × 225 ; Figs. 4, 6, × 50.

Clitoria ternatea, Tephrosia, Pongamia glabra, Dalbergia sissoo, Indigofera and *Teramnus labiales* of the Papilionaceæ.

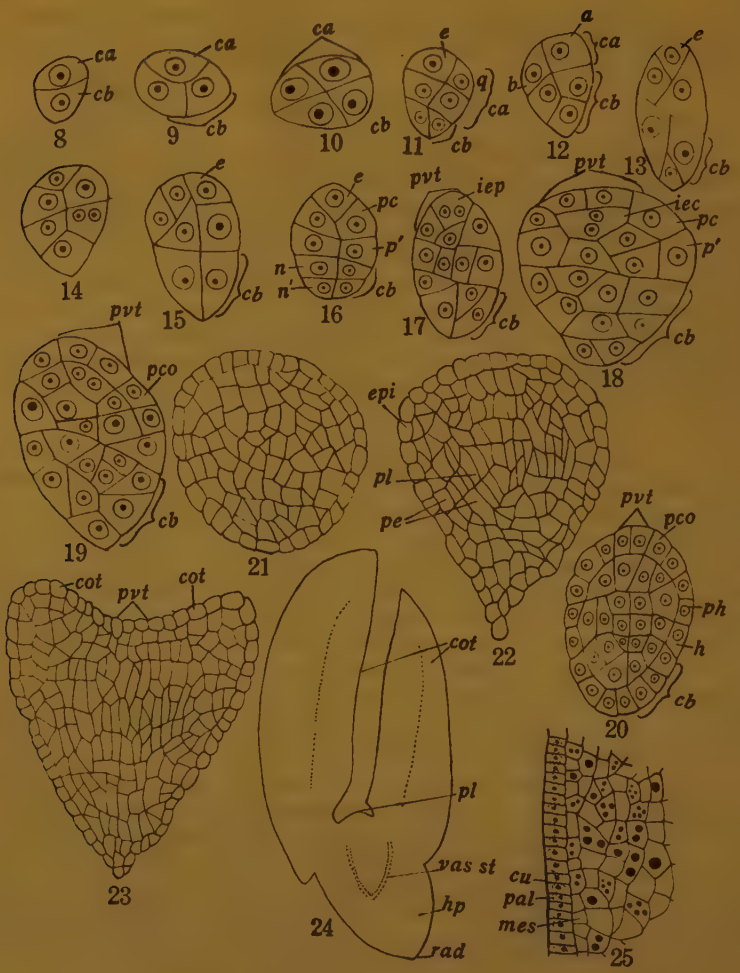
Embryo Development

Neptunia triquetra (Figs. 8–24).—The first division of the oospore is transverse resulting in the formation of the terminal cell, *ca* and the basal cell, *cb* (Fig. 8). The first division in the bicellular proembryo is in the basal cell which is either vertical or oblique (Fig. 9). Next division takes place in the terminal cell which is oblique giving rise to a globular tetrad (Fig. 10) of B_1 of the Series B of Souèges' embryogenic classification. The daughter cells, *a* and *b* of *ca* divide to give rise to quadrants which are disposed in a globular tetrad (Fig. 11). Of the four quadrants, the one which is at the summit functions as an epiphysis initial *e*. This is the upper daughter cell of *a* formed by an oblique division.

The divisions in the two daughter cells of the basal cell, *cb* either take place before those in *a* and *b* or afterwards. These are in variable planes and result in the formation of a massive tissue which does not contribute to the construction of the embryo proper. There is no distinction between this tisssue and the embryo proper when the proembryo becomes massive. From a comparative study of the embryogeny of *Genista tinctoria* (Souèges, 1947 *a*), *Ulex europæus* (Souèges, 1947 *b*), *Sarothamnus scoparius* (Souèges, 1947 *c*), etc. of the Papilionaceæ where the suspensor is massive and is less distinct from the embryo proper, it becomes evident that the massive tissue derived from the basal cell, *cb* in plants studied during the present investigation is homologous to the suspensor.

Figures 14–17 show formation of elements derived from quadrants. The epiphysis initial, *e* divides first tangentially into an upper and lower cell. The upper cell divides vertically and delimits the first initials of the epidermis *iep* of the stem apex, *pvt* (Figs. 17–20). The lower cell gives rise to the initials of the cortex, *iec* of the *pvt*. The quadrants below the epiphysis initial *e* divide transversely or obliquely and form two tiers (Fig. 16). The elements of the upper tier, *pco* give rise to the cotyledonary part *sensu stricto* and the lower tier, *phy* to the hypocotyledonary part, *ph* and the hypophysis, *h*.

The tier, *pco* soon extends to the right and left side of the epiphysis (Figs. 19, 20). The lateral derivations of this tier give rise to the cotyledons while the central part furnishes the plerome of the stem. The transverse or somewhat oblique walls produced in the tier, *phy* result in the formation of two new layers (Fig. 20). The upper layer, *ph* represents the hypocotyledonary part and the lower layer in contact with the tissue derived from the basal cell, *cb*, forms the hypophysis, *h*. Hereafter, it becomes difficult to follow the divisions in regular sequence since they take place in all planes. The resulting massive proembryo assumes either a spherical or pear shape (Figs. 21, 22) and all traces of demarcation between the cotyledonary, hypocotyledonary and hypophyseal parts and the suspensor disappear.



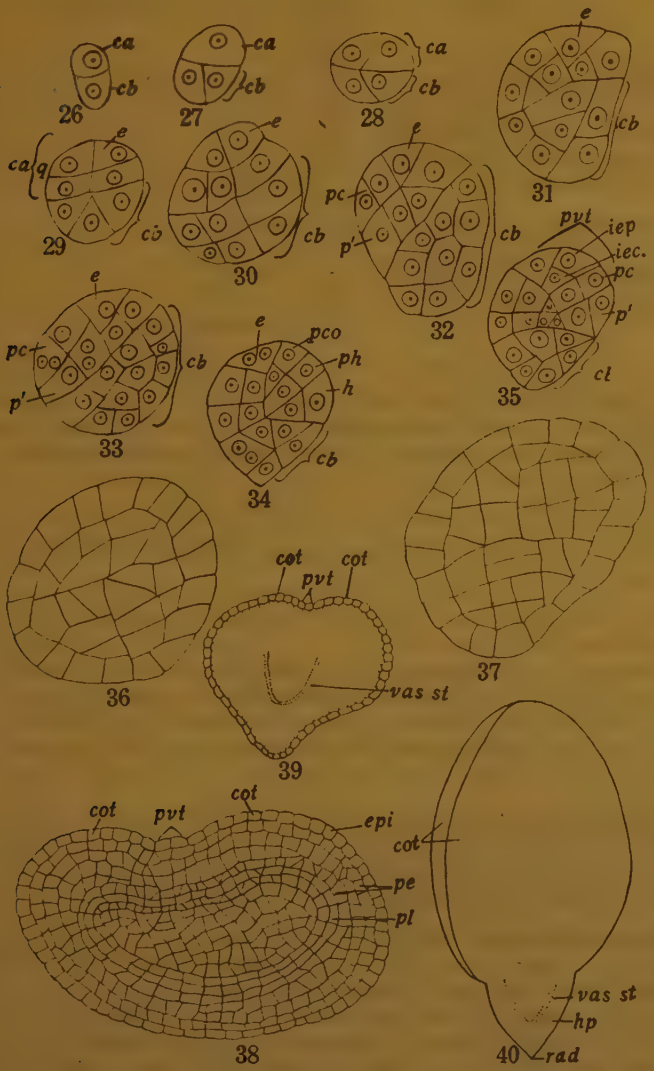
FIGS. 8–25.

Figs. 8–25. *Neptunia triquetra*.—Figs. 8–23. Various stages showing development of embryo. *cot*, cotyledon; *pe*, periblem; *pl*, plerome; *plu*, plumule; *q*, quadrants; *vas st*, vascular strand. For explanation see text. Fig. 24. Mature embryo. *cot*, cotyledons; *hp*, hypocotyl; *rad*, radicle; *vas st*, vascular strand. Fig. 25. L. S. of part of seed-coat. *cu*, cuticle; *mes*, mesophyll; *pal*, palisade. Figs. 8–20, × 500; Figs. 21–23, 25, × 225; Fig. 24, × 50.

Derivation of the embryonic parts is shown in the following table of recapitulation.

I. *First cell-generation* (Fig. 8)

Proembryo of two cells disposed in	..	$ca = pco + pvt + phy + icc + iec$ $cb = s + co$



FIGS. 26–40.

Figs. 26–40. *Prosopis spicigera*.—Figs. 26–39. Various stages showing development of embryo. Legends same as for Figs. 8–25. For explanation see text. Fig. 40. Mature embryo. Legends same as for Fig. 24. Figs. 26–37, × 650; Figs. 38, 39, × 275; Fig. 40, × 25.

II. *Second cell-generation* (Figs. 9, 10)

Proembryo of four cells disposed in two tiers .. { *ca* The destinations of two tiers are the *cb* same as in the preceding generation.

III. *Third cell-generation* (Fig. 11), quadrants

Proembryo of six cells disposed in three tiers .. $\begin{cases} e = pvt \\ c = pco + phy + icc + iec + co \end{cases}$

(Epiphysis, e and three quarants, c below epiphysis and two elements of s) $\begin{cases} \\ cb = s \\ \end{cases}$

IV. *Fourth cell-generation* (Figs. 14–17)

Proembryo of twelve cells disposed in four or five tiers .. $\begin{cases} e = pvt \\ pc = pco + icc \\ p' = phy + iec + co \\ cb = s \text{ (or } n + n' = s) \text{ (Fig. 16)} \end{cases}$

At the fourth cell-generation, initials of epidermis are delimited by tangential divisions taking place in quadrants. When the cotyledonary protuberances appear, the periblem and plerome are differentiated (Figs. 22, 23).

It is somewhat interesting to note that the synergids are seen in the degenerated condition even up to the stage when the proembryo has become 6-celled (Fig. 1).

Prosopis spicigera.—The development of the embryo in this plant is represented in Figs. 26–39. From the study of these figures, it will be seen that its embryogeny is essentially similar to that of *Neptunia triquetra*. After the fourth cell-generation, divisions become irregular and it becomes impossible to follow destinations of the blastomeres. The proembryo becomes massive and assumes a spherical shape (Figs. 36, 37).

The mature embryo in both the plants (Figs. 24, 40), *Neptunia triquetra* and *Prosopis spicigera* is symmetrical and possesses a thick and short radicle, broad hypocotyledonary region and prominent cotyledons enclosing the plumule as in other Mimosaceæ.

From the above observations, it is clear that the embryogeny in the present plants follows the embryonomic type of *Trifolium minus* (Souèges, 1948) in broad outlines, but there is no sharp distinction between the suspensor and the embryo proper. According to Souèges' system of classification, it comes under the First Period of the Megarchtype VI of the Series B (Souèges and Crété, 1952). If Johansen's system (1950) is followed they can be classified under the Onagrad Type, *Trifolium* Variation.

Seed-coat

The structure of the testa of the mature seed of *Neptunia triquetra* (Fig. 25) is essentially similar to that of *Prosopis spicigera* already described by the author (1953). The testa consists of 9–10 layers of the outer integument and 2 layers of the inner integument.

Summary

1. The endosperm follows the Nuclear type of development. At the chalazal end, the endosperm remains free nuclear even as late as the appearance of cotyledons and assumes a tubular form. The organisation of a barrier tissue stops encroachment of the chalazal part of the nucellus by the endosperm.

2. The embryogeny follows the embryonomic type under the First Period of the Megarchtype VI of the Series B of Souèges, or *Trifolium* Variation of Onagrad Type of Johansen.

Acknowledgements

In conclusion, the author wishes to express his gratitude to Prof. R. L. Nirula for guidance, to Dr. René Souèges for going through the manuscript with respect to the embryogeny and offering valuable comments, to Shri U. Mukerjee, Principal, College of Science, Raipur, for facilities, to the Ministry of Education, Government of India and the Education Department, Madhya Pradesh, for research grants.

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EFFECT OF LIGHT EXPOSURE ON THE GROWTH OF *JAPONICA* VARIETIES OF RICE

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In recent years (Kirk and Silow, 1951; Silow and Kjaer, 1952 and Chatterjee, 1953) increasing attention has been paid to the study of *japonica* eco-types with a view to combine the high fertility response of *japonicas* with the tropical adaptation and hardiness characteristic of *indica* varieties. The *japonicas* flower in about 120 days from sowing under Japanese conditions. These varieties behave like short-durationed (*aus*) rice under Indian conditions (Roy and Subramanyam, 1954). The flowering duration of these varieties, when grown under field conditions in normal daylight, has been found to vary between 76–81 days in different varieties as given in Table I. This is probably

Table I

Flowering time and flowering duration of japonica *varieties of rices under field conditions*

Variety	No. of plants raised	Flowering time : flowers fully open on more than 75% of the plants	Flowering duration in days from sowing (12—6—53) to full flowering
Norin 18 ..	576	27–8	77
Asahi ..	144	31–8	81
Rikuu 132 ..	360	27–8	77
Norin 36 ..	144	27–8	77
Gimbozu ..	144	26–8	76
Taichu 65 ..	252	27–8	77

because of the longer day-lengths available during the growth period in the northern latitudes compared with the shorter day-lengths of the tropics during the main paddy season. This reduction in the total availability of daylight causes considerable shortening of their flowering duration, which has been studied by many workers in India as well as elsewhere. In view of this, an investigation was undertaken in this laboratory to induce late flowering by exposure to additional light. This paper presents the results of an investigation on the effect of extra light treatment on the growth and flowering of *japonica* rices.

Six *japonica* varieties, *viz.*, Norin 18, Asahi, Rikuu 132, Norin 36, Gimbozu and Taichu 65, were used in this investigation. Seedlings of each variety were transplanted at the age of 27 days, in pots, 18×18″, each of which was filled with one part of farmyard manure and three parts of soil. Single superphosphate and ammonium sulphate at 4 oz. for each pot was applied as top dressing. There were two pots of three seedlings of each variety. Seedlings established themselves in the course of a week. In the absence of any relevant information on the subject, a three-hour exposure to artificial light from 7 p.m. to 10 p.m. was given to the plants when they were 35 days old and possessed 6 to 8 tillers. For exposure to artificial light, plants were removed to a well ventilated room and kept under an illumination of 500 watt electric bulb at a vertical distance of 12′ from the source of light. The control plants were given normal daylight.

All other conditions except light treatment were kept, as far as practicable, similar in both the cases. The treatment was continued till ears in the control plants had emerged completely.

In this experiment light treatment was applied to the plants when the seasonal day-length was approximately 14 hours. In order to test whether increase in light availability would affect the growth and consequently the yield at harvest, height measurements and yield components *viz.*, ear bearing tiller counts, panicle length, number of grains per panicle, seed sterility and total grain and straw weight of individual plants were recorded.

Height measurement was adopted as the length of the main shoot from its base to the point of exertion of panicle. Measurements were taken when the panicles had completely emerged out of the sheath. The mean values of three plants given in Table III show that as compared with control, increase in light availability has no appreciable variation in the treated plants.

Tiller counts were made after full ear emergence in all the tillers. This includes the main shoot. Compared with control, an appreciable increase in the number of fertile tiller was noticed, as an after-effect of exposure, in plants receiving additional light (Figs. 1 and 2). The mean values given in Table III indicate that treated varieties have shown an increase of 48·8 per cent. to 150 per cent. over the control in the number of fertile tillers. The two varieties Gimbozu and Rikuu 132 have given maximum response.

The criterion of full flowering was taken as the stage at which the lowest spikelet of the ear had become free from the sheath of the flag leaf. For recording the dates of flowering, observations were made each day at 3 p.m. The relevant data of flowering and the number of days from sowing to earing given in Table II indicate that with the same date of sowing there is a marked difference in the date of flowering in these two treatments. A prolongation of 13 to 38 days in the flowering duration has been observed in different varieties. The effect of light treatment in lengthening the flowering duration has been conspicuous in Asahi and Rikuu 132, which were 38 and 29 days, respectively.

TABLE II*

The date of flowering and flowering duration of japonica *rices, sown on* 12–6–1953 *and transplanted on* 19–7–1953, *in pot cultures*

Variety	Control		Treated		Increase in flowering duration of the treated plants in days
	Date of ear emergence	No. of days from sowing to ear emergence	Date of ear emergence	No. of days from sowing to ear emergence	
Norin 18† ..					
Asahi ..	22—8—53	72	29—9—53	110	38
Rikuu 132 ..	26—8—53	76	24—9—53	105	29
Norin 36 .	22—8—53	72	15—9—53	96	24
Gimbozu ..	23—8—53	73	10—9—53	91	18
Taichu 65 ..	15—8—53	96	28—9—53	109	13

* Each value represents the average of three plants.

† All the plants transplanted were not true to type and hence were excluded from the study.

It is of interest to record here that Taichu 65 takes only 77 days to flower from sowing under field conditions (Roy and Subramanyam, 1954). The control plants of this variety in this investigation, have taken 96 days from sowing to flowering. While reasons for this deviation are not known, it is likely that better cultural methods obtained under pot conditions might have contributed towards lengthening the flowering duration of this variety.

The period required to ripen from the date of flowering to full maturity in both the treated and control plants is approximately the same, 35 days.

Panicle length was measured from the base of the commencement of spikelet to the tip of the ear. Table III shows that the treatment causes no increase in ear length.

The number of grains per panicle was counted along with the number of sterile spikelets in each plant. The percentage of grain setting and sterility was calculated. Table III shows that in treated plants there was an increase of 1 to 14 per cent. of grain setting whereas the seed sterility was reduced by the same percentage in different varieties.

The yield data were obtained from the weight of total grains and straw of individual plants in both the control and light treated plants. The ears were dried in the sun and the grains weighed. Likewise straw

TABLE III*

The growth performance of japonica *rices*

	Control					Treated				
	Asahi	Rikuu 132	Norin 36	Gimbozu	Taichu 65	Asahi	Rikuu 132	Norin 36	Gimbozu	Taichu 65
Height in inches ..	43·50	55·40	44·20	42·70	52·30	44·50	53·20	42·60	55·10	48·10
No. of ear bearing tillers ..	41·00	34·00	31·00	18·00	34·00	61·00	76·00	59·00	45·00	66·00
Percentage increase over control in the number of fertile tillers						48·76	123·53	90·32	150·00	94·12
Panicle length in inches ..	6·30	4·00	7·30	6·30	6·30	6·20	4·30	7·60	6·30	6·30
Percentage grain setting ..	46·00	75·00	52·00	48·00	39·00	47·00	84·00	63·00	62·00	46·00
Percentage seed sterility ..	54·00	25·00	48·00	52·00	61·00	53·00	16·00	37·00	38·00	54·00
Percentage reduction in sterility ..						1·00	9·00	11·00	14·00	7·00
Weight of grains in gms. ..	26·36	40·88	24·15	29·53	29·68	53·61	98·29	49·87	36·56	70·18
Percentage increase in grain weight over control						129·49	140·44	106·50	23·89	136·46
Straw weight in gms. ..	133·65	105·85	95·50	72·58	102·98	224·68	273·69	168·37	162·75	249·88
Percentage increase over control in straw weight						68·11	158·56	22·34	124·24	142·65

*Each value represents the average of three plants.

weight was also recorded. The data presented in Table III show that all the varieties receiving extra light, except Gimbozu responded very highly to this treatment. The increase in grain yield has been more than 100 per cent. over control with the exception of Gimbozu which showed 23·89 per cent. increase over the control. Similarly Rikuu 132, Gimbozu and Taichu 65 showed more than 100 per cent. increase over control in starw weight.

Summary

An exposure of 3 hours additional light to *japonica* rice varieties lengthens the flowering duration from 13 to 38 days, but has no marked effect on panicle length and plant height. It increases the number of fertile tillers, total grain, straw weight, grain setting and decreases seed sterility by the same percentage, 1 to 14 per cent.

From the results obtained in this work it may be concluded that shorter day light conditions of the tropics are mainly responsible for inhibiting the growth performance of *japonica* rices and better results may be obtained by exposure to additional light.

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Explanation of Photographs

Fig. 1. A Japanese rice, Rikuu 132. The plant on the left has received normal illumination, while the one on the right has received light for extra 135 hours.

Fig. 2. A Japanese rice, Rikuu 132. A. Ears from a plant that has received normal light. B. From a plant that has received extra light.

FIG. 1

FIG. 2

OBSERVATIONS ON THE VASCULAR ANATOMY OF THE STEM OF *BARRINGTONIA ACUTANGULA* GÆRTN.

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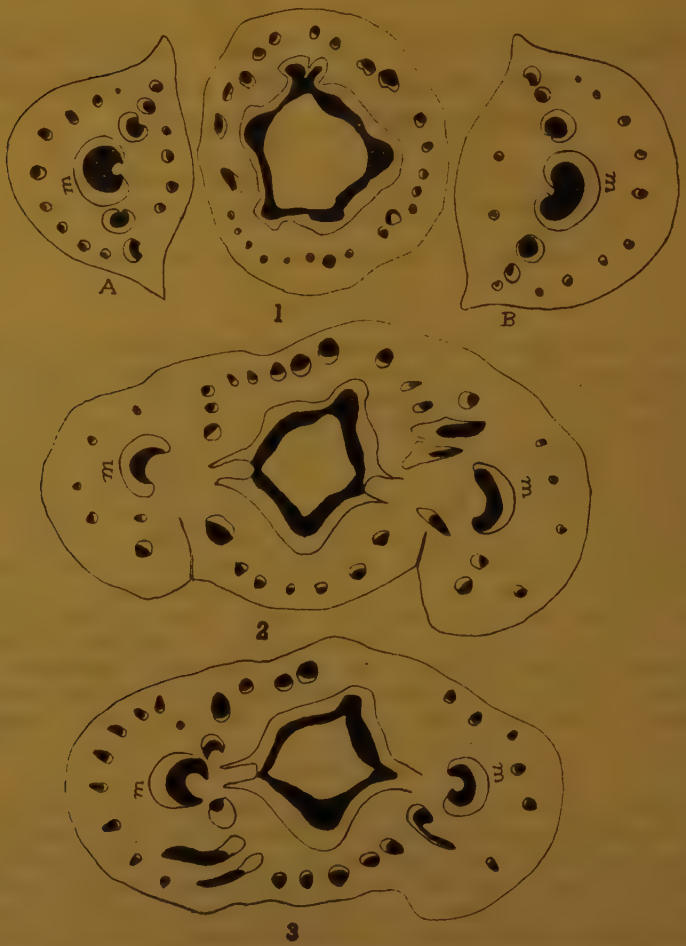
(Received for publication on January 10, 1954)

Introduction

The vascular anatomy of *Barringtonia acutangula* Gærtn. (*Lecythidaceæ*) presents certain interesting features. A transverse section of the young stem shows, besides a continuous vascular ring within the pericycle, a large number of vascular bundles in the cortex. The striking feature about these cortical bundles is that they are orientated in an inverted fashion, that is, with the xylem outside and phloem inside. The presence of cortical bundles has been observed in many diverse families as may be observed from a perusal of Solereder's Systematic Anatomy of the Dicotyledons (Boodle, 1908). Writing about the vascular anatomy of *Lecythidaceæ*, Solereder has referred to the occurrence of inverted cortical bundles and has even given some account of the nature of their relation with the axial vascular system, but no detailed study of the subject has been made so far. The following observations have been made with a view to trace the course of the cortical bundles in relation to the axial vascular system in *Barringtonia acutangula*.

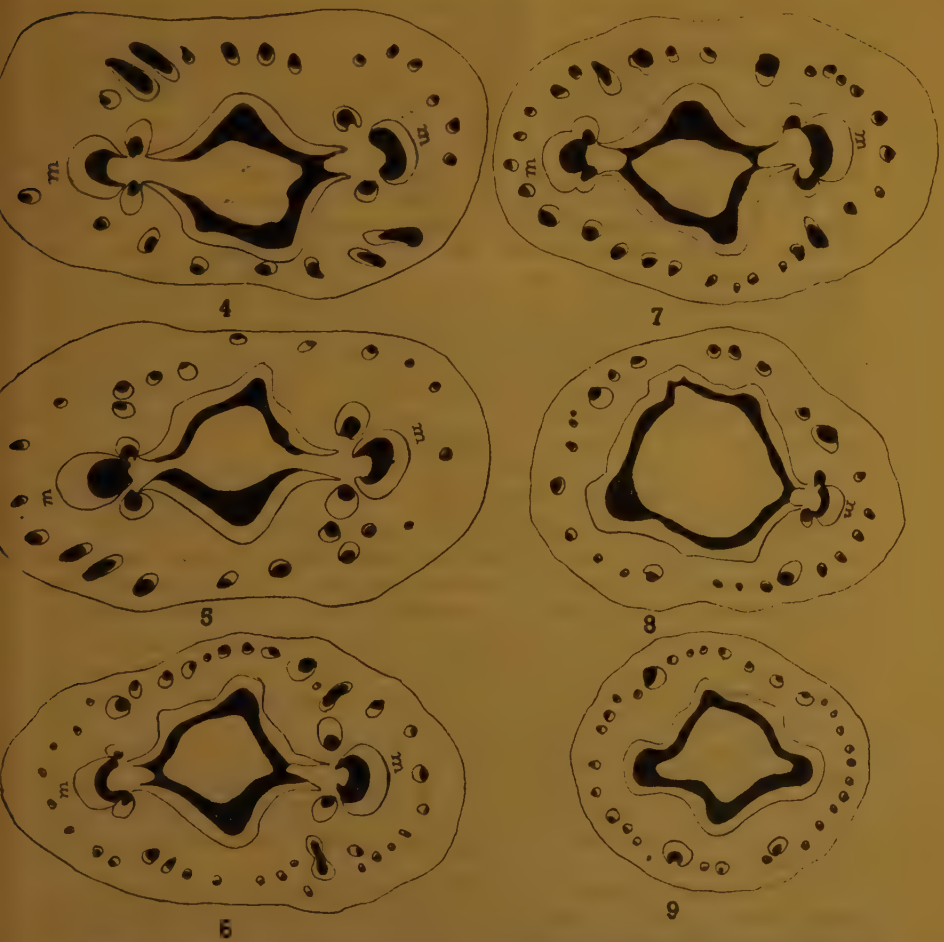
Observations

Figures 1 to 9 represent a series of transverse sections of the stem from a region just above a node to the internode immediately below. To begin with, the central axial portion contains in the middle a ring of vascular tissue with the phloem and xylem normally orientated. Outside the ring are found a number of cortical bundles with the relative position of their phloem and xylem reversed. On either side of the axis, there are petioles of the opposite leaves (A and B in Fig. 1) showing three vascular systems: (1) An arc of 5–7 bundles in the middle, with a large central bundle and progressively smaller lateral bundles; (2) a row of small vascular bundles on the ventral side; and (3) an arc of somewhat larger bundles on the dorsal side. The vascular bundles of the first two systems are normally orientated, but those on the dorsal side are inverted (Fig. 1). In Fig. 2, the petioles are seen fusing with the stem. The vascular cylinder of the latter now shows conspicuously the foliar gaps. At the next stage (Fig. 3), the large horse-shoe-shaped middle bundle of the middle vascular system of the petioles with the two lateral bundles, one on either side, approach the axial vascular system, considerably narrowing down the foliar gaps. At the same time, the remaining lateral bundles of the middle system of the petioles undergo a twist through 180° and become the



FIGS. 1–3. Fig. 1. Transverse sections of the axis and two petioles, A and B, of the opposite and decussate leaves. Fig. 2. Approximation of the middle vascular arc of the petiole towards the vascular ring of the axis. Note the characteristic leaf-gap. Fig. 3. The middle three bundles of the petiole (*m*) almost in the process of union with the axial vascular ring ; the other bundles of the petiole in different stages of inversion during their entry into the cortex and before constituting the inverted cortical vascular bundles. × 28.

cortical bundles of the lower internode on the sides of the main stem corresponding to the edges of the middle vascular arc of the petioles. Figures 3 to 6 show these bundles in different stages of torsion. Going downward further, the fusion of the vascular cylinder of the main axis with the three central bundles of the middle vascular system of the petiole is seen to be almost complete (Fig. 7). It may be pointed out here that the description of Solereder (Boodle, 1908) does not seem to accord with the present observations when he says that it is only the



FIGS. 4-9. Figs. 4–6. Sections of the node showing further stages of the fusion of the middle leaf-traces (*m*) with the axial vascular ring. Fig. 7. The three large leaf-traces (*m*) in the act of fusion on either side of the main vascular ring. Fig. 8. Fusion on the left is complete while on the right the identity of the leaf-traces is not yet lost. Fig. 9. Section of the internode showing the completion of this process ; the bulges in the main vascular ring on the right and left indicate the regions of its union with the leaf-traces. × 28.

central bundle of the principal vascular system of the petiole that joins the vascular cylinder of the main axis and all the laterals enter the stem as cortical bundles. From the figures given here it must be evident that not only the central bundle but also the two laterals, one on either side of it, join the axial cylinder. Recent work on the anatomy of the Dicotyledons (Metcalfe and Chalk, 1950) has not clarified this point. The originally dorsal vascular bundles of the petiole already orientated in an inverted manner descend down without any change

into the lower internode of the main axis and directly form the cortical bundles on the same side as and peripheral to the middle vascular system of the petiole which by now has become hardly distinguishable except as a bulge from the vascular cylinder of the main axis because of its complete fusion with the latter (Figs. 8 and 9). The few bundles constituting the ventral vascular arc of the petiole join the lateral vascular bundles of the middle arc in their downward course towards becoming the inverted cortical bundles. Figure 8 represents the region just below the node. It may be considered almost as a transition between the node and the internode and this fact is evident because one of the petiolar vascular systems has almost lost its identity through fusion with the vascular system of the main axis, while the other petiolar vascular system is in the process of undergoing such a fusion (Fig. 8, *m*). Figure 9 shows the structure of the internode proper where no longer the distinction between the vascular bundles of the main axis and the petioles can be made out.

Conclusions

From the foregoing description of the course of the vascular bundles in the stem and petioles, four points can be made out:—

(1) Three bundles of which the middle is the largest, branch off from the vascular cylinder of the main axis into the petiole and constitute the central portion of the middle vascular arc of the petiole.

(2) The majority of the cortical bundles of the stem traverse the petiole on the lower side and constitute its dorsal vascular system. These bundles show inverted orientation whether they be in the stem or in the petiole.

(3) Some of the cortical bundles of the stem traverse through the middle of the petiole and become the lateral bundles of the middle vascular arc of the petiole. These bundles have inverted disposition while they are in the stem, but become normally placed as they emerge into the petiole.

(4) A small number of these lateral bundles of the middle arc while traversing the petiole may become split and constitute the ventral arc of poorly developed bundles.

Acknowledgments

The authors wish to express their grateful thanks to Dr. T. C. N. Singh, M.Sc., D.Sc., F.B.S., Professor and Head of the Department of Botany, for his valuable suggestions and helpful criticisms.

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SPORE GERMINATION AND REGENERATION IN *PHYSCOMITRIUM PYRIFORME* (L.) BRID.

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(Received for publication on June 5, 1954)

The first account of spore germination in *Physcomitrium pyriforme* is credited to Goebel (1889) who noted the development of an insignificant rhizoidal system from protonema and the formation of both thick vigorous branching green threads and thin pale green filaments—the latter with straight cross walls. Rabe (1905) observed that the spore on germination produced first the rhizoidal filament and then the germ tube from the opposite side. According to Schoene (1906) the germination in this species is of the usual *Funaria* type. Meyer (1947) reports the same feature for *P. turbinatum*.

In the present investigation spore germination in *P. pyriforme* has been studied from multispore cultures on Knop's half per cent. solution, on sterilised canal soil and on filter papers in sterilised petri dishes and monospore mounts in hanging drops of Knop's solution. The spores were collected from Gauhati (India) during autumn, 1952 and experiments are performed, identical to those of Paul and Meyer (1949) on *P. turbinatum*, to demonstrate the capacity of regeneration from setæ which had been cut into several parts. They show some variations from those described by them. Leaves removed from healthy gametophytes were cultured on moist filter papers in sterilised petri dishes for regeneration.

Spore Germination

The spores are spherical, about 33 μ in diameter and brown. The exine bears minute conical thickenings (Fig. 1). Germination occurs within 4 days, but all the spores from a given capsule do not germinate simultaneously. On coming in contact with moisture they absorb water and swell up. This causes the rupture of exine at one or two points or it may break into a few pieces (Figs. 2 and 3). From one side is given off a papillate protrusion full of chloroplasts, this develops into a filament. At this stage the germ cell undergoes a transverse division followed by protrusion of a rhizoid—a thinner filament with scanty or no chloroplasts. It is given off either from the same side, laterally (Figs. 5 and 8), or from the opposite side (Figs. 4 and 7). Occasionally two non-chlorophyllous broad filaments are given off from the opposite sides and later one of them behaves as a rhizoid and the other develops chloroplasts (Fig. 6). Both filaments later become branched and behave in the usual manner. It is of interest that the

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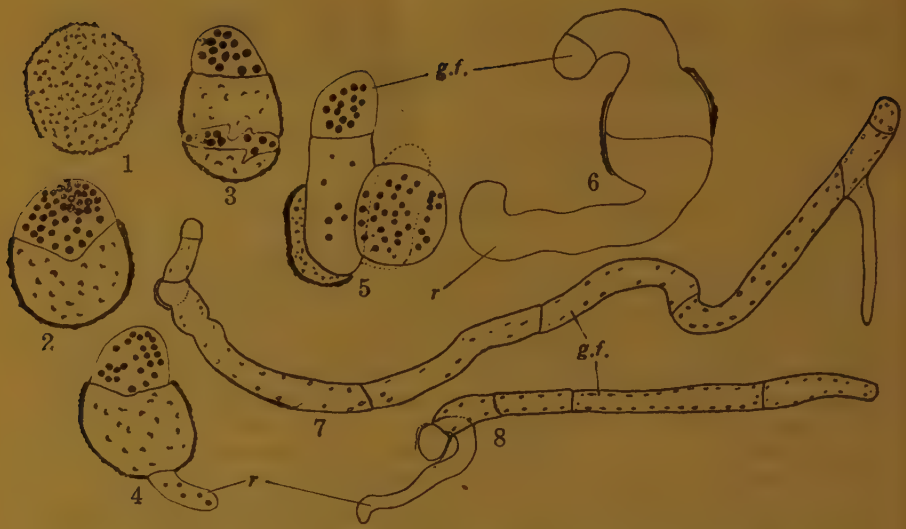

FIGS. 1–8. *Physcomitrium pyriforme*. Fig. 1. Spore. Figs. 2–6. Stages in emergence of green filament (*g. f.*) and rhizoid (*r*). Figs. 7–8. Young protonema filaments. Figs. 1–6, × 425; Figs. 7–8, × 45.

emergence of the rhizoid and the germ papilla is not usually simultaneous. Occasionally the first rhizoid may not appear even though the green filament may attain a length of 1·5 cm.

The rhizoidal filaments are thin-walled and colourless when young, but become thick-walled and light-brown in older regions of the protonema. Their cross walls are oblique and the branches are always thinner than the mother filaments. Older rhizoids are without contents, while the younger ones show the presence of variable number of degenerating chloroplasts. It is not, however, uncommon to find green filaments also exhibiting rhizoidal features. This may be due to extreme moist conditions and low illumination. Since cultures placed in optimum conditions of illumination and moisture never showed gradations between the two except that *very rarely* the rhizoidal filaments behaved as green filaments and even produced a green leafy shoot. There might appear, thus, some justification in regarding green filament and rhizoid not 'fundamentally different (Paul, 1903)'. Artificial cultures on aqueous media with different salt concentrations did not show any fundamental difference in behaviour but such protonemata showed marked difference in habit when compared to those grown on solid media and on canal soil. However, there does not exist any correlation between pH of the substrate and growth of the protonemata. They are as luxuriant on artificial soil cultures as in nature. It is evident that moisture exerts a major influence on growth and development of leafy shoot. This supports the observations of Meyer (1942) on *P. turbinatum* and *Funaria hygrometrica*. Under continued

excessive humid conditions and low illumination protonemata become long, thin, with scanty chloroplasts [showing an approach to *P. turbinatum* and *F. hygrometrica* (Meyer, 1948) protonemata which lose chlorophyll when placed in darkness for a long time] in each cell and with most of the filaments behaving as rhizoids. Some of the pre-

FIGS. 9–10. *Phycomitrium pyriforme.*—Regeneration from injured (Fig. 9) and uninjured (Fig. 10) leaves, × 15.

cociously growing ones come above surface and at once undergo a few transverse divisions followed by delimitation of an apical cell to form a young plant. Rarely being crowded again the segments of the apical cell bud off into fresh filaments. Young leafy shoots in such environments form numerous protonemata from their bases, leaf margins and very rarely from shoot surface.

Treboux (*vide* Meyer, 1948) states that light was not necessary for germination of spores of *P. pyriforme*. On the contrary it is noted that these spores do not germinate when placed in dark even upto 3 months. However, addition of 0·5–1% of glucose to 1% Knop's solution caused swelling and rupture of spore within a month and a half. This as Schulz (*vide* Meyer, 1948) believed might be due to accumulation of sugar; they do not develop further unless immediately placed in optimum light conditions.

It is interesting to note that all the 4 types of spore germination namely: (i) only the green filament develops from the spore; (ii) the

formation of rhizoid was followed by the formation of filaments; (iii) simultaneous formation of rhizoid and the green filament; (iv) the formation of green filament followed by the formation of rhizoid; described for *Funaria hygrometrica* (Schoene, 1906) and *P. turbinatum* (Meyer, 1947) occur in this species. But the number of any particular group depends largely on the conditions of media and the environment. The green filaments have an apical growth and a definite apical cell. A vertical division in the latter results in branching. Rarely the germ cell may also undergo a vertical division to form two germ papillæ, each of which later develops into a green filament. The formation of a shoot from the protonema is simiar to that of *Funaria* and other mosses.

Regeneration

For a study of regeneration the setæ were removed from plants by cutting at the base just above the foot and at the upper end just below the apophysis of the capsule. Later cultures of whole setæ with capsules, without capsules, half setæ cut from the apophysis end and from the foot region and each part of the setæ cut out into eight equal parts were tried. Each culture contained 100 segments. The lowest percentage of regeneration was shown by the segment of the setæ with capsule attached to it. A comparison of percentages of regeneration is tabulated below:—

Portion used	Total No. of segments	Total No. of segments showing regeneration
Whole setæ segments ..	100	65
Top segments of half cut setæ (from the apical region)	100	52 (in one case from the basal region)
Basal segments of half cut setæ (from the apical region)	100	38
⅛th segment of setæ (from the apical region) ..	100	20
Capsules with setæ ..	100	4

Protonemata develop from the epidermis as well as from the inner tissue and are more numerous from the latter as in *P. turbinatum* (Paul and Meyer, 1949). Their development from the basal ends of the setæ except when with capsule, was almost insignificant in these experiments.

Regeneration was quick and profuse in leaves with an injured base (Fig. 9)—protonemata developing both from the intact marginal cells, from the injured regions and from the surface cells (either towards the base or apex of the leaf). From entire leaves regeneration was comparatively less but of the same order (Fig. 10). Whereas fresh protonemata may arise from any region of the leaf, in all cases they are more frequent from the basal region and were never observed aris-

ing from the apex of the leaf. When defoliated plants were grown submerged protonemata were formed from the leaf scars as in *P. turbinatum* (Meyer, 1942), but contrary to what happens in that species, in *P. pyriforme* such protonemata are more profuse than those developing from injured plants. Plants with injured stems, in moist chambers, also produced protonemata, though rhizoid formation was more profuse.

Summary

P. pyriforme shows all the four types of spore germination described for *Funaria hygrometrica*. The condition of medium and illumination determine to a large extent the prevalence of a particular type over the other. Regeneration from the various regions of the segmented setæ and the leaves is described. In the former the protonemata develop from both the internal and the epidermal tissue of the cut end of the setæ and in the latter from the margin and from the surface of the leaf. Germination and regeneration did not occur in dark.

I am indebted to Prof. William C. Steere for kindly going through this note. He drew my attention to, and kindly lent some of Dr. S. L. Meyer's publications on the subject. Thanks are due to Dr. A. Noguchi for identifying the species.

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CURVULARIA SPECIES FROM DISCOLOURED GRAINS FROM HYDERABAD-DN.

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(Received for publication, on June 2, 1954)

It is known from the results of investigations of various authors that the discoloration of grains of food crops is gaining importance due to the fact that it is increasingly prevalent in Hyderabad-Dn., Madhya Pradesh, Bengal and in other cereal growing areas of the country.

Investigation (1952–53) at the Plant Pathology Laboratory, Botany Department, Osmania University, into the discoloration of the rice grains collected from the various rice growing areas of Hyderabad, showed, that out of 800 discoloured grains collected, on isolation, over 60% were infected with *Curvularia* spp. Further, collections of infected sorghum and bajra earheads also revealed the presence of *Curvularia* spp. It is quite likely that species of *Curvularia* cause much damage to cereal grains.

In this paper it is proposed to give a brief account of the various species of *Curvularia* responsible for the discoloration of the grains.

(1) *Curvularia lunata* (Wakker) Bœdijn .. Paddy.

(2) *Curvularia pallescens* Bœdijn .. .. Paddy.

(3) *Curvularia geniculata* (Tracy and Earle) Bœdijn .. .. Paddy.

(4) *Curvularia affinis* Bœdijn .. .. Paddy.

(5) *Curvularia spicifera* (Bainier) Bœdijn .. Paddy.

(6) *Curvularia pennisetti* (Mitra) Bœdijn .. Bajra.

Symptoms on Paddy

The fungi affect the inflorescence and discolor the entire ear. In all cases glumes are affected and become brown to black. The fungi extend into the grain to a considerable depth, as a result of which the grain tend to become soft and chalky; sometimes the pathogens induce emptiness of the grain. The leaves also get affected, gradually showing dark-brown or brick-red appearance from the tip downwards; the disease is locally known as *Ettakarogum*. In each case, both sides of the leaf show dark sclerotia, which perhaps survive in the soil after harvest and thus attack the next crop.

Curvularia lunata (Wakk.) Bœd. Syn. *Acrothecium lunatum* Wakker

Bœdijn in *Bull. Jard. bot. Buitenz.*, ser. 3, XIII, 127 (1933). Saccardo, *Syll. Fung.* XIV, p. 1089 (1899). Mason, Annotated

FIG. 1. A. *Curvularia lunata*. B. *Curvularia pallescens*. C. *Curvularia geniculata*. D. *Curvularia affinis*. E. *Curvularia spicifera*. F. *Curvularia penniseti*.

account of fungi received at the Imperial Bureau of Mycology, list ii, Fasc. 1, p. 5 (1928). Waheeduddin, S., *Annual Report Dep. Agric.*, Hyderabad (1940).

Conidiophore simple, unbranched, septate, straight or bent, dark brown, sometimes knotted at the tip, 80–250 $\mu \times 3 \cdot 6$–$4 \cdot 8 \mu$.

Conidia arranged in thick panicles at the top of the conidiophore in varying numbers, conidia bent to one side only, dark olive brown, 2–3 septate, rounded at the tip with central enlarged, darker, and paler apical and basal cells; basal cell having a scar showing the point of attachment. Conidia, 21×11 ($14–26 \cdot 4 \times 8 \cdot 4–13 \cdot 2\,\mu$).

Curvularia pallescens Bœd.

Bœdijn in *Bull. Jard. bot. Buitenz.*, Ser. 3, XIII, p. 127 (1933). Marchionatto, *Rev. Argent. Agron.* 10, 2, 114–16 (1944).

Conidiophore simple, dark olive brown, branched and septate with slightly swollen apex. Conidia in clusters and sometimes in thick panicles at the tip of the conidiophore, usually three-septate, dark-brown, mostly straight, slightly curved, the middle two cells larger and darker in coloration than the apical and basal cells. Basal cell crucible-shaped, with a scar showing the point of attachment. End cells distinctly hyaline, measuring 24×9 ($18–30 \times 7 \cdot 2–10 \cdot 8$) μ.

Curvularia geniculata (Tracy and Earle) Bœdijn. Syn. *Helminthosporium geniculatum* Tracy and Earle, 1896.

Bœdijn in *Bull. Jard. bot. Buitenz.*, Ser. 3, XIII, p. 129 (1933). Mason, Annotated account of Fungi received at the Imperial Bureau of Mycology, list ii, Fasc. 1 (1928). Tracy and Earle, *Bull. Torrey. bot. Cl.*, XXIII, p. 207 (1896).

Conidiophore simple, hyaline, septate, irregular; conidia arranged in thick panicles (2–10 conidia), usually 3-septate, straight or slightly curved (geniculate), the third cell from the base larger, more dilated and darker in colour than the rest, end cells distinctly hyaline, basal cell with a scar showing the point of attachment. They measure $25 \cdot 0 \times 10 \cdot 7$ ($20 \cdot 4–33 \cdot 6 \times 8 \cdot 4–12 \cdot 0$) μ. 75 per cent. of the paddy material collected from district Nizamabad on isolation gave mostly 4-septate forms which have been found to agree quite well with the description and measurements given by Bœdijn for *C. geniculata*.

Curvularia (*?*) *affinis* Bœd.

Bœdijn in *Bull. Jard. bot. Buitenz.*, Ser. 3, XIII, p. 130 (1933).

Conidiophore simple, slender, hyaline, septate, straight or curved and knotted at the tip; conidia attached at the tip of the knotted conidiophore, 2–6 in number. Conidia 4-septate, constricted at each septum, brown, the second and third cell from the base darker and larger than the rest. Sometimes only the middle cell is larger and dark coloured, conidia slightly curved at this cell but mostly straight, colour of the conidia hyaline or light brown but not deep olive brown. Basal cell much longer than the apical ones, apical and basal cells hyaline, measuring $30 \cdot 0–46 \cdot 0 \times 8 \cdot 4–13 \cdot 2\,\mu$ average $35 \cdot 0 \times 9 \cdot 6\,\mu$. From the measurements and the type and coloration of the conidia it is clear that it does not agree either with *C. fallax* Bœd. or *C. affinis* Bœd. As such it has been tentatively identified as *Curvularia affinis* Bœd.

Curvularia spicifera (Bainier) Bœdijn. Syn. *Helminthosporium tetramera* Mckinney.

Haynes, J., *Proc. Roy. Soc.*, N.S.W. 70, pp. 378–91 (1937). Thomas, K. M., *Adm. Rep. Mycologist*, Madras, 52–56 (1936). Ganguly, A. K. & Ganguly, D., *Sci. & Cult.*, 6, 424–25 (1941).

Conidiophore simple, dark olivaceous brown, irregular, unbranched, straight or curved and septate at regular intervals; conidia 20–40 arranged in clusters on the conidiophore, predominantly 4-celled, middle two cells broader dark olive brown with a thin epispore wall. Conidia straight tapering towards the end cells; scar present showing the point of attachment. Conidia measuring $20{\cdot}4–43{\cdot}2 \times 7{\cdot}2–12\,\mu$. This species lacks the enlarged central cells which is characteristic of *Curvularia*. Germination by apical cell. Sclerotial production not observed in culture.

Curvularia penniseti (Mitra) Bœdijn.

M. Mitra, *Mem. Dept. Agric. India Bot.* Series 2, pp. 57–74 (1921).

Symptoms.—The symptoms of the disease in Bajra earhead externally appear as that of smut fungus, few grains here and there becoming sooty black in color, but on examination it is found to be a species of *Curvularia*. The symptoms produced by this fungus are different from the one described by Mitra (1921) for *Acrothecium penniseti*.

Conidiophore simple, erect and dark-brown, septate at regular intervals. Conidia in clusters of 3–5, 2–3 septate, straight or slightly curved, dark dirty brown with central enlarged and darker coloured cell and paler end-cells. Slight constriction at each septum, measuring $24{\cdot}0–42{\cdot}0 \times 12{\cdot}0–19{\cdot}2\,\mu$. Wall of the conidia dark and thicker than any of the other known species.

Acknowledgments

We wish to record our thanks to Prof. M. Sayeeduddin for his encouragement during this investigation.

THE DEVELOPMENTAL MORPHOLOGY OF *CHARA ZEYLANICA* WILLD.*

By V. S. Sundaralingam**

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(Received for publication on April 24, 1954)

The morphology of the vegetative and reproductive structures of the Characeæ has been the subject of study by various authors ever since 1815.

A good historical account of the work of the earlier authors is given by Allen (1888), Migula (1897) and Karling (1926). Most of these accounts are very meagre and superficial and often inaccurate.

Braun (1852) was the first to give an accurate account of the structure of the different parts of the plant in the group. He laid the foundation for the present method of classification of the members of the group based on the structure of the plant. Sachs (1882) studied in detail the developmental morphology of the vegetative structures in *Chara fragilis* and of the sexual structures in *Nitella flexilis*. In 1882 Allen studied the details of the development of the cortex in some of the American species of *Chara*. Giesenhagen (1896, 1897, 1898) was the first to investigate in a very thorough manner the origin and development of the different parts of the vegetative thallus in the Characeæ. He has given a good account of the developmental morphology of *Nitella cernua*, *N. gracilis*, *N. syncarpa*, *Chara stelligera*, *Tolypella intricata* and *Lychnothamnus alopecuroides*. He also studied the vegetative reproduction in the group by bulbils. Ernst (1904) studied the stipular leaves of *Nitella hyalina*. In (1906) Kuczewski carried out a thorough investigation of the developmental morphology of the vegetative structures in *Chara delicatula*. Drew (1926) studied the adventitious branch development from the 'leaf' of *Nitella opaca*. In 1929, Walter studied the development of the sexual organs, the fertilisation and the zygote development in *Nitella hyalina*, *N. syncarpa* and *N. batrachosperma*.

No work has been done in India so far on the developmental morphology of any member of the Characeæ. All the work done in India has been only of a systematic nature. So a detailed study of the developmental morphology was taken up of *Chara zeylanica* Willd. (Text-Fig. 99), a species which grows most commonly almost everywhere in the country in tanks, ponds, pools and marshes.

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Methods

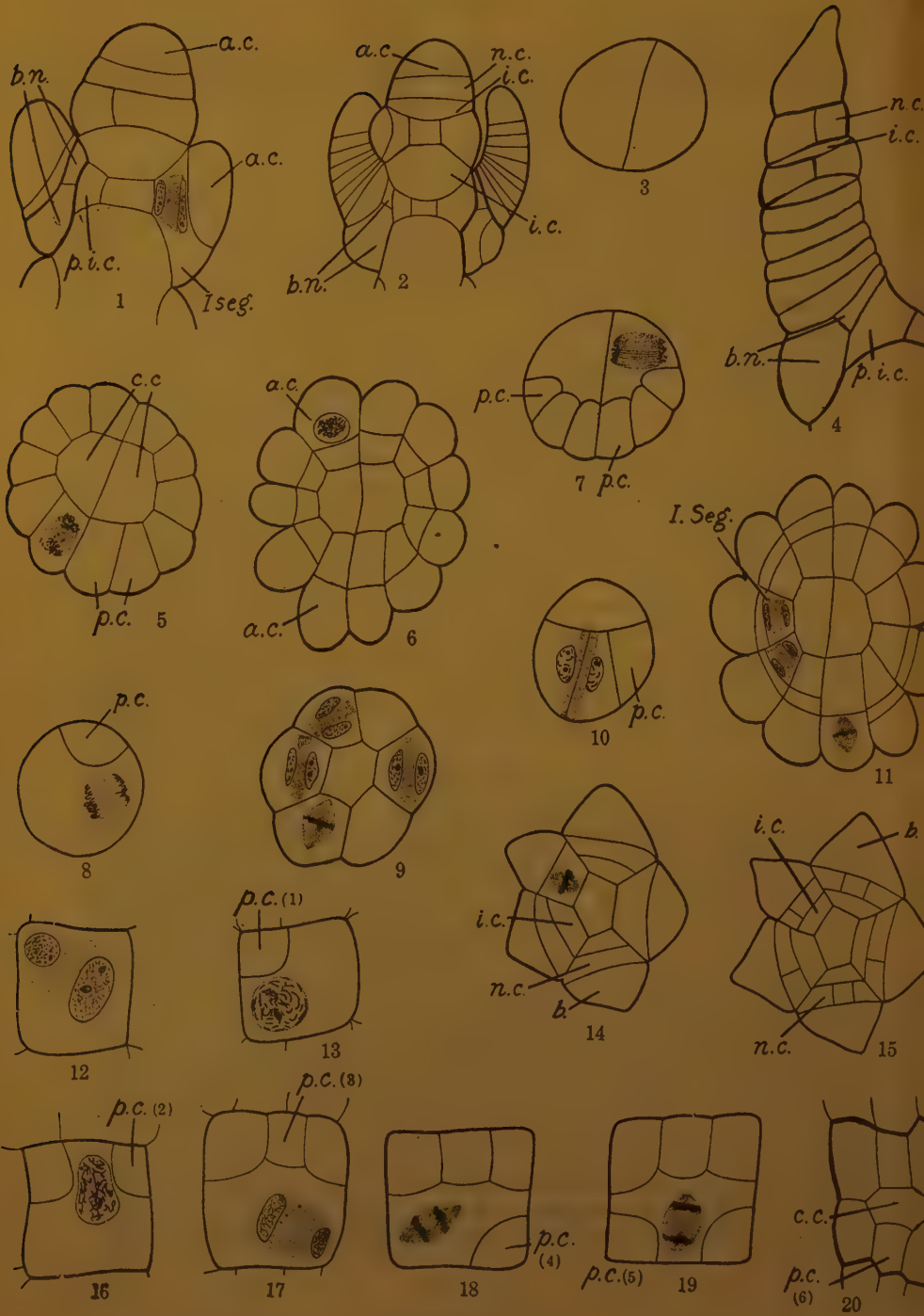
The material was fixed in Nawaschin's fluid. Sections were cut 5–10 μ in thickness, stained in Haidenhain's iron-hæmatoxylin and mounted in canada balsam.

Development of the Main Axis

The main stem shows an unlimited growth. Growth is by means of a dome-shaped apical cell, which cuts off continuously transverse segments below (Pl. X, Figs. 2, 3; Text-Figs. 1, 2, *a.c.*). Each of these segments then divides into an upper biconcave and a lower biconvex cell (Text-Fig. 2). The lower cell does not divide any further, but elongates and forms the internodal cell (Text-Fig. 2, *i.c.*). The upper cell is the nodal initial which by a series of vertical curved walls forms the stem node. The node consists of a central pair of cells surrounded by a number of peripheral cells, (Text-Fig. 5, *c.c.*, *p.c.*). The nodal initial cell first of all divides into two semi-circular cells by means of a vertical wall, the "halving wall" (Text-Fig. 3). In each of these two cells a semi-circle of peripheral cells is cut off by vertical curved septa, the sequence of the cutting off of the cells being alternately to the right and to the left of the original halving wall (Text-Figs. 7, 43). The successive divisions are in a plane intersecting the preceding plane of division (Text-Fig. 7). The first cell is cut off to the right of the halving wall and the second to its left. The cutting off of the cells in the two semi-cells progresses in opposite directions (Text-Figs. 7, 43) and finally closes the ring with the result that a complete circle of cells is formed enclosing two cells in the centre, which are the remnants of the two original semi-cells (Text-Fig. 5). The number of peripheral cells in the stem node of *Chara zeylanica* is usually twelve, though occasionally it may be eleven (Text-Figs. 6, 11) or ten.

Branchlets

Each of the peripheral cells gives rise to a branchlet by a series of divisions. To start with, each peripheral cell (branchlet initial cell) becomes protruded and then divides into an outer and an inner cell by a periclinal wall (Text-Figs. 1, 6). The outer cell becomes the apical cell of the branchlet. The inner cell divides periclinally into an outer cell (*basal nodal cell*) and an inner cell (*primary internodal cell*) (Pl. X, Fig. 1; Text-Figs. 1, 11, *I seg.*). The apical cell, by further cutting off a series of segments in the same manner as the apical cell of the main axis, gives rise to a branchlet (Text-Figs. 2, 4). After it has cut off seven to twelve segments, it assumes an elongated conical shape and ceases to divide any further (Text-Fig. 4). At this stage the whole branchlet is in the form of a pile of flat disc-shaped segments which narrow gradually upwards, surmounted by the large conical apical cell at the top and with the basal nodal cell and the primary internodal cell at the bottom (Text-Fig. 4). Each of these disc-shaped segments divides by a transverse wall into a biconcave upper cell (*the nodal initial of the branchlet*) and a biconvex lower cell (the internodal cell of the branchlet (Text-Fig. 4, *n.c.*, *i.c.*). The division of these segments into



TEXT-FIGS. 1–20 .

TEXT-FIGS. 1–20. *Chara zeylanica.* Figs. 1, 2. L.S. of stem apex with dome-shaped apical cell. Fig. 3. T.S. of stem nodal initial with the halving wall. Fig. 4. L.S. of young branchlet showing the formation of the nodal and internodal initials. Figs. 5–7. Formation of peripheral cells in the stem node and the divisions of the peripheral cells to form the branchlets. Figs. 8–10. Formation of the peripheral cells in the branchlet node. Figs. 12, 13, 16–20. T.L.S. (Tangential Longitudinal Section) of the cortical nodal cell showing stages in the development of the branchlet cortex. Figs. 11, 14, 15. Division of the peripheral cells to form the bract and the nodal and internodal cells. *a.c.*, apical cell; *b.*, bract; *b.n*, basal node; *c.c.*, central cell; *i.c.*, internodal cell; *n.c.*, nodal cell; *p.c.*, peripheral cell, *p.i.c.*, primary internodal cell; *I seg.*, first segment of branchlet. (Figs. 1–11, 14, 15, ×300; rest, × 420.)

the nodal and the internodal cells is basipetal and starts first in the uppermost segments and progresses downwards to the lower segments (Text-Fig. 4). In this connection it may be mentioned that Kuczewski (1906) found the division of the segments into nodal and internodal cells to be basifugal in *Chara delicatula*, whereas Sachs (1882) found it to be basipetal in *Chara fragilis*. The internodal cells elongate in the same manner as those of the main axis, only their elongation is very limited.

The nodal initial, by a series of vertical curved septa, forms a node consisting of a number of peripheral cells enclosing a single cell in the centre (Pl. X Fig. 6; Text-Figs. 8–10). Unlike in the main axis, no halving wall is formed in the nodal initial during the development of the branchlet node. The cutting off of the peripheral cells in the branchlet nodes starts as soon as each of the primary segments of the branchlet divides into the nodal and the internodal cells (Text-Fig. 4). The number of peripheral cells cut off in the nodes of the branchlet is not the same throughout, but increases gradually from four near the top to eight near the bottom. The first peripheral cell is cut off towards the main axis (Text-Figs. 4, 8). The second and the third cells are cut off to the right and to the left of the first peripheral cell in a plane intersecting the plane of division of the first cell (Text-Fig. 10). The remaining cells are cut off likewise alternately to the right and to the left until the circle of peripheral cells is completed (Pl. X, Fig. 6; Text-Fig. 9). Soon after this the branchlet gradually covers itself with a cortex. Each peripheral cell of a branchlet node divides periclinally into an outer and an inner cell (Pl. X, Fig. 6; Text-Fig. 9). The inner cell divides again periclinally into an outer and an inner cell so that a row of three cells is formed (Text-Fig. 14). Of these the outermost cell is the bract cell (*b*), the middle cell is the nodal cell (*n.c.*) and the innermost cell is the internodal cell (*i.c.*) (Text-Figs. 14, 15, 21). The outermost cell grows out into a bract, which usually takes an upward direction (Text-Figs. 15, 21, 23, 24 *b*). The nodal cell cuts off, by a series of divisions, six peripheral cells round a central cell (Text-Figs. 12, 13, 16–20). The first peripheral cell is cut off at the top left corner (Text-Figs. 12, 13, *p.c.*) the second at the top right corner (Text-Fig. 16, *p.c.* 2) and the third in between the above two so that an upper row of three cells is formed (Text-Fig. 17, *p.c.* 3). The fourth cell is then cut off at the bottom right corner (Text-Fig. 18, *p.c.* 4) the fifth at the bottom left corner (Text-Fig. 19, *p.c.* 5) and the sixth between the fourth

and the fifth cells (Text-Fig. 20, *p.c.* 6). Thus a single cell is left in the centre surrounded by six peripheral cells (Text-Fig. 20).

The internodal cells of the branchlet then begin to elongate. As they elongate, the peripheral cells also elongate adhering closely to the internodal cell and form the cortex of the branchlet. The lower peripheral cells give rise to the descending rows of cortical cells, while the upper peripheral cells give rise to the ascending rows of cortical cells, so that the two oppositely growing rows of cortical cells meet in the middle of the internode of the branchlet (Pl. X, Fig. 7; Text-Fig. 24). In most species of *Chara*, the lowest segment of the branchlet has a cortex consisting of the descending rows only. But in the present *Chara* (*Ch. zeylanica*) the lowest segment is ecorticate (Text-Fig. 25). This is due to the fact that the descending row of cortical cells in this node is generally poorly developed (Text-Fig. 25, *l.p.c.*). In a longitudinal section of the branchlet only a slight development of the descending rows of cortical cells is seen.

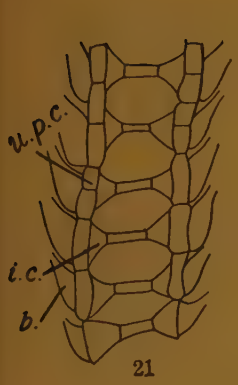
The central cell is very narrow (Text-Fig. 20, *c.c.*) and in longitudinal section appears squarish or oblong (Pl. X, Fig. 7; Text-Fig. 24, *c.c.*). This cell, in course of time, presumably by the pressure exerted all round by the growth of the upwardly and the downwardly growing cortical rows, appears to be very much crushed. It then gradually degenerates and finally gets obliterated. In old branchlet nodes the internodal cell (Text-Fig. 23, *i.e.*) grows larger, and protrudes inwards crushing and obliterating the central cell of the branchlet node (Text-Fig. 23, *c.c.b.n.*). This peculiar in-growth does not appear to have been recorded previously.

Another interesting feature is that the bract, which develops before the formation of the middle nodal cell, occupies the whole area of the anterior face of the above nodal cell. Even after the development of the node and the cortical cells, each bract in a longitudinal section is seen to be in contact with the three upwardly growing cortical cells and the three downwardly growing cortical cells and the central cell (Pl. X, Fig. 7; Text-Figs. 21–24).

Braun (1852) states that in the case of *Chara fragilis*, during the development of the branchlet cortex, a definite central cell is formed. But Kuczewski (1906) states that no central cell is formed. The writer examined some material of *Ch. fragilis*, sent by G. O. Allen to Prof. M. O. P. Iyengar regarding this point. In surface view of the cortex, it looks as though no central cell is present. But a careful examination, at a lower focus, clearly reveals the existence of a central cell, as recorded by Braun. In the case of *Chara zeylanica*, which has a triplostichous branchlet cortex, the central cell is very definitely seen (Pl. X, Fig. 7; Text-Figs. 23, 24, *c.c.*).

Basal Node of the Branchlet

The structure and the development of the basal node are very characteristic and differ from those of the remaining nodes of the branchlet. Before the upper segments of the branchlet divide into



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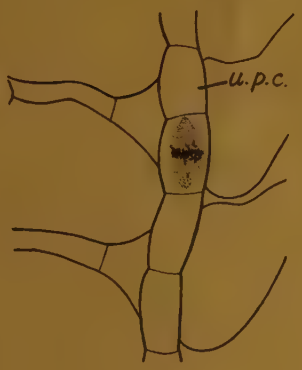

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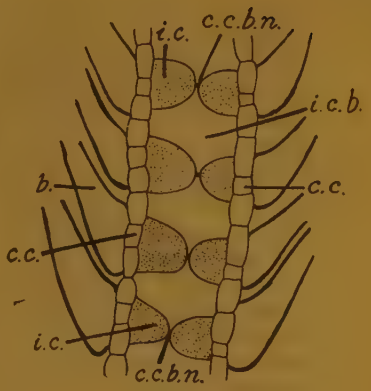

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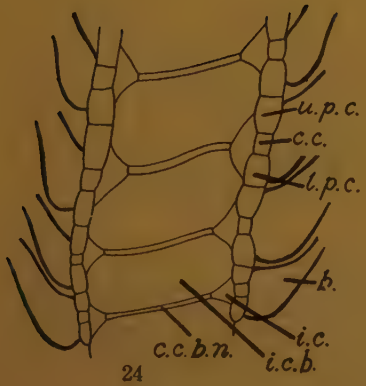

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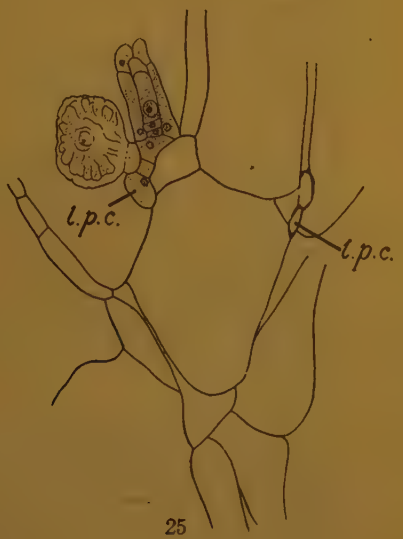

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TEXT-FIGS. 21–25

TEXT-FIGS. 21–25. *Chara zeylanica.* L.S. of branchlet showing stages in the development of the bract and the branchlet cortex. Fig. 21. L.S. of branchlet nodes prior to the formation of the cortex. Fig. 22. Cortical nodal cell of the branchlet cutting off the lower peripheral cell. Fig. 23. Branchlet nodes showing the peculiar enlargement of the innermost cells (*i.c.*). Fig. 24. Branchlet nodes showing the upper and the lower peripheral (cortical) cells and the central cell. Fig. 25. Lowermost branchlet node showing the poorly developed downward cortex (*l.p.c.*) and the consequent ecorticate condition of the lowermost internode of the branchlet. *b.*, bract cell; *c.c.*, central cell; *c.c.b.n.*, central cell of branchlet node; *i.c.*, internodal cell; *i.c.b.*, internodal cell of branchlet; *l.p.c.*, lower peripheral cell. *u.p.c.* upper peripheral cell. (Figs. 21–24, × 160. Fig. 25, × 76.)

the nodal initials and the internodal cells, the basal node initial begins to cut off cells (Text-Figs. 2, 4, *b.n.*). The details of development of the basal node are very complicated. Most of the authors on Characeæ do not give the details of development of this basal node. Giesenhagen (1896, 1897, 1898) and Kuczewski (1906) are the only authors who give a detailed account of its development. The main features of development in the present *Chara* are somewhat as follows:—

The basal node initial is more oval than round in cross-section and somewhat narrower towards the side facing the main axis (pointed by the arrow) (Text-Figs. 26, 27, 29). It develops into the basal node by cutting off four peripheral cells around a single central cell (Text-Figs. 29, 31). The first peripheral cell is cut off on the side of the main axis (Text-Figs. 27, 28). The second cell is cut off to the right and the third cell to the left, the plane of division of each cell intersecting the plane of division of the first cell (Text-Figs. 27, 29). The fourth peripheral cell is cut off by a wall intersecting the walls of the second and the third cells so that the node is fully formed with a single cell in the centre surrounded by four peripheral cells (Text-Figs. 29, 31).

In a median longitudinal section of the stem node with the plane of division passing through the axial region of the branchlet, the basal node shows (1) the first, peripheral cell; (2) the second or the third peripheral cell; and (3) fourth peripheral cell, with the central cell in the middle (Text-Fig. 30). In a transverse section of the stem node with the plane of division passing through the axial region of the branchlet (*i.e.*, in a plane at right angles to that of the previous division), the second and the third peripheral cells are seen enclosing the central cell (Text-Fig. 44). From an examination of the sections it would appear that the newly formed lateral walls of the second and the third peripheral cells are disposed obliquely. They appear to bend towards each other in the middle of the node and the lower and hinder parts of each of the two walls show a curvature towards the periphery of the node.

Of the four peripheral cells formed the first peripheral cell forms the upwardly growing cortical lobe of the upper stem internode (Text-Figs. 32–34), the second and the third peripheral cells by further divisions give rise to the stipulodes (Text-Figs. 44–47, 51) and the fourth peripheral cell forms the downwardly growing cortical lobe of the lower stem internode (Text-Figs. 32–34).

Regarding the origin of the basal node of the branchlet, Migula (1897) states that each peripheral cell of the stem node divides into two cells, of which the outer cell divides again by a transverse wall into an outer apical cell and an inner cell from which is developed the the basal node of the branchlet. But Kuczewski (1906), on the other hand, states that each peripheral cell of the stem node divides by a periclinal wall into an outer and an inner cell and the inner cell divides again by a transverse wall into two cells of which the upper cell becomes the basal node of the branchlet and the lower one forms the primary internodal cell. In the present *Chara*, the origin of the basal node is more in agreement with the observations of Kuczewski

than with those of Migula. Here each peripheral cell of the branchlet divides periclinally into two cells of which the lower cell becomes subdivided again into an upper nodal cell (*the basal nodal cell*), and a lower internodal cell (*the primary internodal cell*), of the branchlet (Pl. X, Fig. 1; Text-Fig. 1 *I seg.*).

Stipulodes

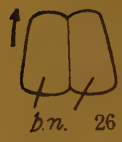
In the present *Chara*, two pairs of stipulodes are formed at the base of each branchlet (Text-Fig. 100, *u.s.*, *l.s.*). In each pair, one is longer and is directed upwards and the other is shorter and is directed downwards. These two pairs of stipulodes are formed by the second and the third peripheral cells, respectively, of the basal node of the branchlet. The course of development of the stipulodes is as follows: The second and the third peripheral cells protrude outside and each of these peripheral cells then divides by a periclinal wall into an outer and an inner cell (Text-Figs. 45–47). The outer cell elongates and becomes the upper stipulode (Text-Fig. 47, *u.s.*). The inner cell divides by an oblique wall into two cells the outer one of which grows downward and becomes the lower stipulode (Text-Figs. 46, 51, 48, *l.s.*) while the inner one does not develop any further. Thus each basal node of the branchlet produces two pairs of stipulodes.

Stem Cortex

The stem is covered by a well-developed cortex. The development of the cortex takes place in the following manner. The basal node of each branchlet is also responsible for the formation of the stem cortex. While the second and the third peripheral cells give rise to the two pairs of stipulodes, the first and the fourth peripheral cells give rise to the stem cortex. The first peripheral cell of the basal node of the branchlet grows elongating upward, while the fourth peripheral cell grows elongating downwards. These cortical lobes extend upwards and downwards closely pressed against the elongating stem internode.

The upwardly growing cortical lobal initial (*i.e.*, the elongating first peripheral cell) divides into an upper and lower cell (Text-Fig. 32, *u.c.* 1 and *u.c.* 2). The upper cell becomes the apical cell of the lobe (Text-Fig. 33, *a.c.*). The lower cell divides by a vertical wall into two cells. Each of these two cells then divides into two cells by a transverse wall (Text-Fig. 33) so that a group of four cells is formed below the upper cell. The apical cell (upper cell) cuts off a series of transverse segments generally about 6–7 (Text-Fig. 34), each of which divides into a bi-concave nodal cell and a bi-convex internodal cell (Text-Fig. 35). This division into nodal and internodal cells starts first in the oldest segment and progresses towards the apical cell (Text-Fig. 35).

The downwardly growing cortical lobal initial (*i.e.*, the elongating fourth peripheral cell) divides transversely into an apical and a lower cell (Text-Fig. 32, *l.c.* 1 & *l.c.* 2). The apical cell behaves in the same manner as that of the upwardly growing cortical lobe. It cuts off



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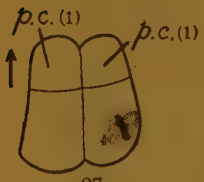

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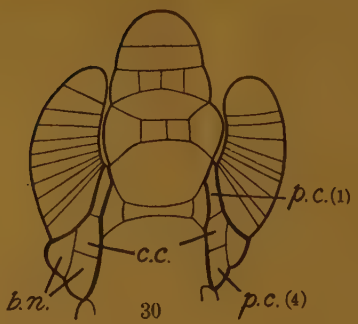

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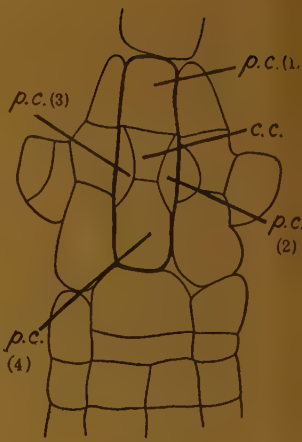

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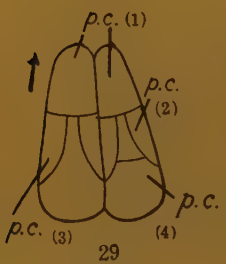

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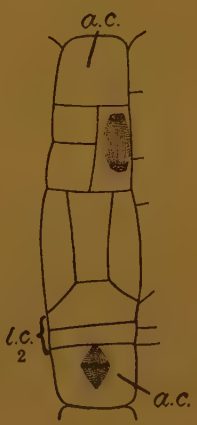

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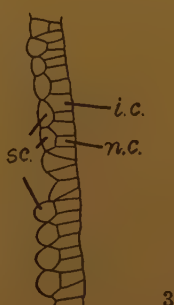

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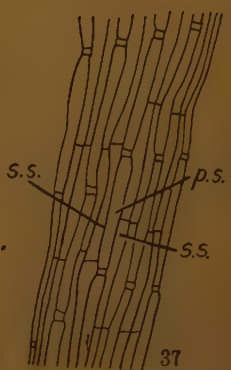

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TEXT-FIGS. 26–37

TEXT-FIGS. 26–37. *Chara zeylanica.* Stages in the development of the basal node and the stem cortex. Figs. 26–29. T.S. of two adjacent basal nodes showing the formation of the four peripheral cells (arrow points towards the stem apex). Fig. 30. Stem apex showing the basal nodes in L.S. with the peripheral cells and the central cell. Figs. 31–35, 37. T.L.S. of the developing stem cortex from the basal node (a single basal node is shown in thick lines). Fig. 36. L.S. of stem internode along with the cortex. *a.c.*, apical cell; *b.n.*, basal node; *c.c.*, central cell; *i.c.*, inter nodal cell; *l.c.*, downward cortical series; *n.c.*, nodal cell; *p.c.*, peripheral cell; *p.s.*, primary cortical series; *s.c.*, spine cell; *s.s.*, secondary cortical series; *u.c.*, upward cortical series. (Figs. 26–29, 31, 33, × 327; rest, × 180.)

a series of transverse segments, generally about 6–7, each of which divides into a nodal and an internodal cell (Text-Figs. 34, 35). But the behaviour of the lower cell here (Text-Fig. 32, *l.c.* 2) is quite different from that in the upwardly growing cortical lobe. This cell divides by a transverse wall into two cells of which the upper becomes a node and the lower remains undivided (Text-Fig. 33, *l.c.* 2).

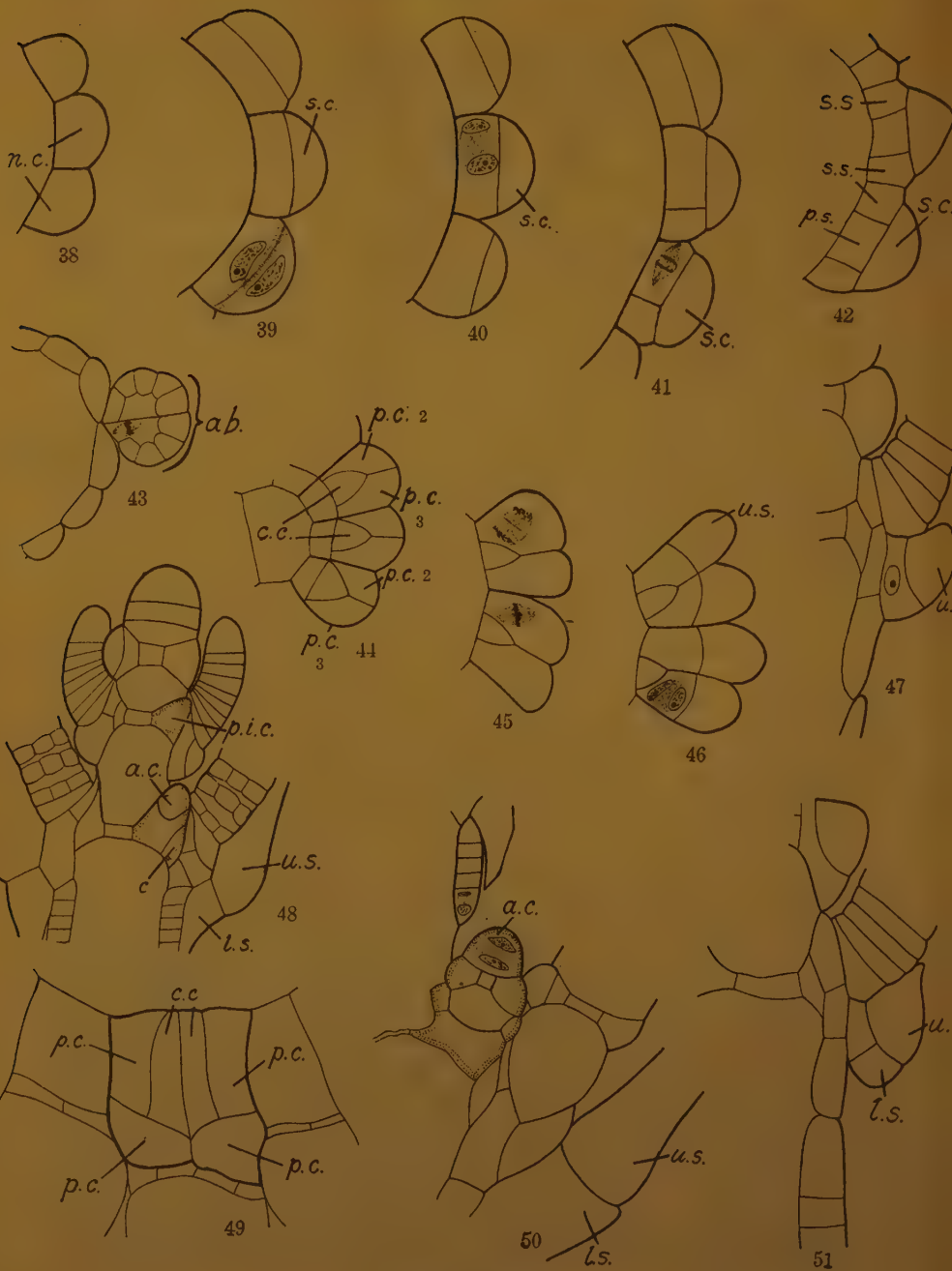
The ascending and descending cortical lobes are not directly one above the other, but alternate with one another and so dovetail with one another (Pl. X, Fig. 5; Text-Figs. 32, 34, 35). This alternation is due to the fact that each branchlet is shifted to the right by half a breadth from that of the preceding whorl. The ascending and descending cortical lobes appear to meet in the middle of the stem node, but since in the earlier stages, *i.e.*, before the elongation of the stem internode, the successive nodes are in contact with each other at their periphery, they are actually in contact with one another throughout their development.

It was pointed out above that the segments cut off by the apical cell of the cortical lobe, divide into a nodal and an internodal cell. Each nodal cell of the cortical lobe is roughly semi-circular in cross-section with the flat side closely adhering to the stem internode (Text-Fig. 38, *n.c.*). It divides into an outer and an inner cell by a wall parallel to the stem internode (Text-Figs. 36, 39). The inner cell then divides by two parallel vertical walls in a plane at right angles to the first wall into a central and two lateral cells (Text-Figs. 40–42).

With the growth of the stem internode, the internodal cells of the cortical lobe also elongate. Along with the latter, the two lateral cells of the cortical node also grow and elongate longitudinally.

Since in their earlier stages the successive nodal cells of the cortical lobe are in contact with each other at their periphery, the two lateral cells of the successive nodes of the cortical lobe are placed one over the other longitudinally and meet about the middle of the internodal cell of the cortical lobe. Thus in a fully developed cortex, each cortical lobe comes to have three parallel rows, the middle row made up of alternate long (internodal) and short (nodal) cells (primary cortical series Text-Fig. 37, *p.s.*) and the adjacent row on either side made up of longitudinally elongated cells (secondary cortical series Text-Fig. 37, *s.s.*). Thus the cortex is triplostichous (Text-Fig. 37).

The outer cell cut off by the cortical nodal initial grows out into a single fairly long spine and the spines of the ascending cortical lobe have a tendency to incline upwards and those of the descending lobe



TEXT-FIGS. 38–51

TEXT-FIGS. 38–51. *Chara zeylanica.* Figs. 38–42. T.S. of the stem internode showing stages in the development of the stem cortex. Fig. 43. T.S. of the internode of the main stem with T.S. of the developing node of the axillary branch (*a.b.*) alongside. Figs. 44–47. T.S. of the stem node showing the stages of development of the stipulodes from the basal node. Figs. 48–50. L.S. of the upper portion of the main axis showing stages in the development of the axillary branch (stippled) out of the primary internodal cell. Fig. 49. T.S. of the first node of the axillary branch with the four peripheral cells surrounding a central pair of cells. Fig. 51. L.S. of the stem node showing the formation of the upper (*u.s.*) and the lower stipulode (*l.s.*). *a.b.*, axillary branch; *a c.*, apical cell; *c.c.*, central cell; *l.s.*, lower stipulode; *n.c.*, nodal cell; *p.c.*, peripheral cell; *p.i.c.*, primary internodal cell; *p.s.* primary cortical series; *s.c.*, spine cell; *s.s.*, secondary cortical series. *u.s.*, upper stipulode. (Figs. 38–42, 44–47, 51, × 313; rest, × 180.)

downwards. Again the spines nearer the stem nodes are often more developed than those towards the middle of the stem internode. The cortical rows follow the torsion of the stem, which could be observed very clearly in the mature cortex.

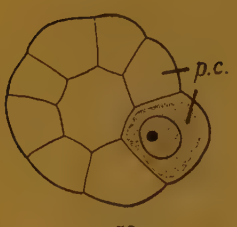
The behaviour of the basal node of the first branchlet of the stem is different from that of the basal node of the remaining branchlets. The first peripheral cell of the basal node of this first branchlet (oldest branchlet) does not produce an ascending cortical lobe owing to the development of a branch in the axil of the branchlet (Pl. X, Fig. 4; Text-Fig. 50). Hence the total number of ascending cortical lobes is one less than the total number of descending cortical lobes and in a fully developed cortex, since each cortical lobe consists of three parallel rows of cells, the total number of rows of ascending cortical cells is three less than the total number of rows of descending cortical cells. No gap is formed, however, in the ascending cortex since the ascending cortical lobes proceeding out of the branchlet on either side of the first branchlet (*i.e.*, the second and the third branchlets) close up the gap (Text-Fig. 43).

Regarding the development of the stem cortical node, Migula (1897) states that the two lateral cells are cut off first and the spine cell only later. But Kuczewski (1906) on the other hand states that the spine cell is cut off first and the lateral cells only later. In the case of the present *Chara zeylanica*, the writer found that the spine cell was cut off first and the lateral cells were cut off later.

BRANCH

One branch is formed at each node. It arises from the axil of the oldest branchlet, *i.e.*, the first branchlet. The primary internodal cell of the first branchlet (*i.e.*, the cell below the basal node of the branchlet), is the starting point of the branch (Pl. X, Fig. 2; Text-Fig. 48. *p.i.c.*).

The primary internodal cell of the first branchlet grows upwards with a broadly rounded top (Text-Fig. 48). It cuts off by an oblique wall a small cell close to the basal node of the branchlet (Text-Fig. 48, *c*). This lower cell does not grow any further. The upper larger cell gives rise to the branch. This cell by a curved transverse wall divides into an upper and a lower cell (Text-Fig. 48). The upper cell becomes



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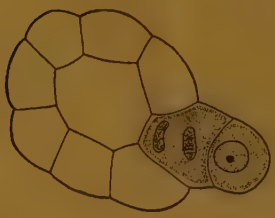

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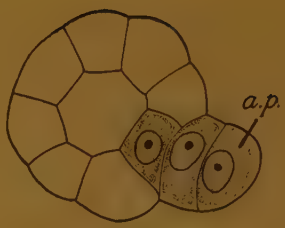

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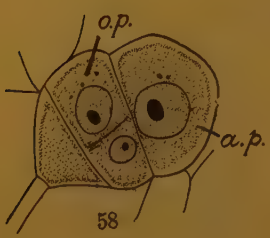

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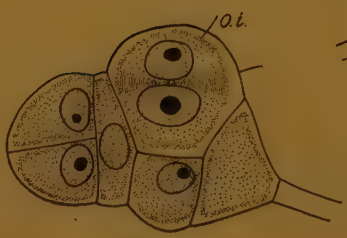

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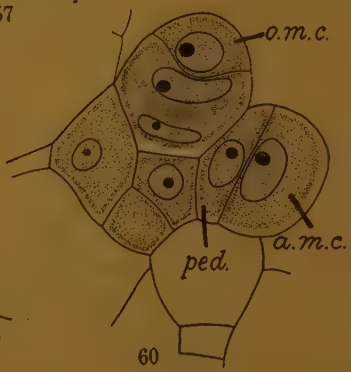

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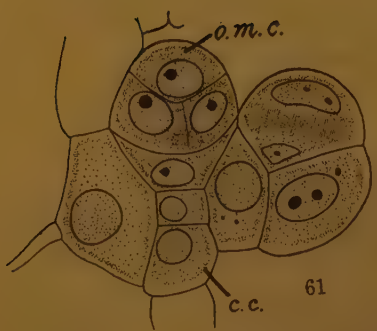

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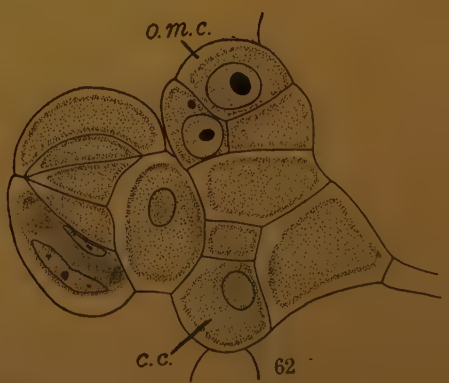

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TEXT-FIGS. 52–62

TEXT-FIGS. 52–62. *Chara zeylanica.* Stages in the development of the antheridium and the oogonium. Figs. 52–54. T.S. of the branchlet node showing stages in the division of the peripheral cell (stippled) into a row of three cells. Figs. 55–57. The same stages shown in L.S. Fig. 58. L.S. of branchlet node showing the differentiation of the oogonial primordium (*o.p.*). Fig. 59. The oogonial primordium dividing to form the oogonial mother-cell. Fig. 60. The cell below the oogonial mother-cell (*o.m.c.*) dividing to form the lower internodal cell and the upper nodal cell. Note also that the antheridial primordium has cut off a pedicel cell (*ped.*) at its base. Fig. 61. L.S. of young antheridium and oogonium. Note one of the octants of the antheridium undergoing periclinal division and also the nodal cell of the oogonium dividing to form the node. Fig 62. Later stage of the same with the antheridium in the sixteen-celled stage. *a.m.c.*, antheridial mother-cell; *a.p.*, antheridial primordium; *c.c.*, cortical cell; *o.m.c.*, oogonial mother-cell; *o.p.*, oogonial primordium; *p.c.*, peripheral cell; *ped.*, pedicel cell. (Figs. 52–54, ×325; rest, × 450.)

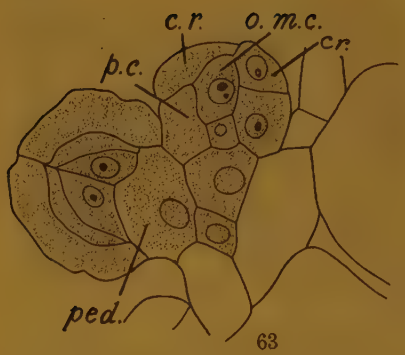
the apical cell of the branch and the lower cell forms its first node by a number of vertical walls (Text-Fig. 49). The upper cell cuts off a series of segments in the same way as the apical cell of the main stem (Pl. X, Fig. 4; Text-Fig. 50). Each segment, as in the main stem, divides by a transverse wall into a node and an internode and the further stages of development are very similar to those of the main stem.

The details of development of the first node of the branch are slightly different from those of the ordinary stem node. As during the development of the stem node, a halving wall is formed, but instead of 10–12 peripheral cells, only 4 peripheral cells are cut off round the two central cells (Text-Fig. 49). The remaining nodes of the branch however, like the nodes of the stem, form 10–12 peripheral cells round the two central ones (Text-Fig. 43). The peripheral cells of the first node of the branch unlike those of the remaining nodes of the branch do not give rise to any further structures.

As already stated, the internode of the main stem above the stem node from which a branch is produced lacks one ascending cortical lobe, since, owing to the growth of the branch in the axil of the first branchlet, the upwardly growing cortical lobe initial of the basal node of the same is very much compressed and presumably its further growth is suppressed (Pl. X, Fig. 4; Text-Figs. 48, 50).

In the first internode of the branch, the upwardly growing cortical portion is wanting, since no ascending cortical lobes are formed by the basal node of the branch. Only a downwardly growing cortical portion is produced from the first free node of the branch.

Regarding the origin of the branch, Migula (1897) states that in *Chara*, the branch takes its origin from a peripheral cell of the basal node of the first branchlet (*i.e.*, from the cell which gives rise to the ascending cortical lobe in the other branchlets). But Kuczewski (1906) states that the primary internodal cell of the first branchlet is the starting point of the branch. In the case of the present *Chara* (*Chara zeylanica*), the writer finds that the branch takes its origin definitely from the primary internodal cell as stated by Kuczewski.



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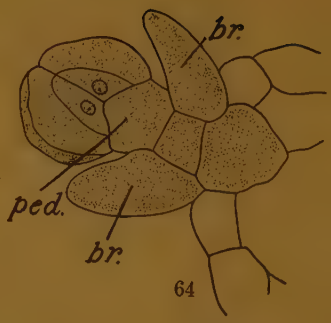

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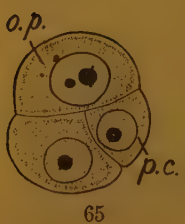

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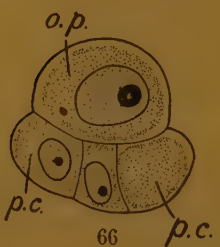

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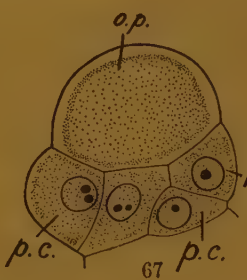

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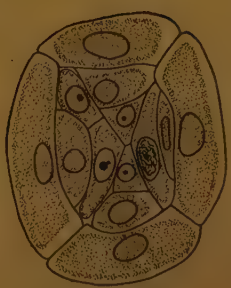
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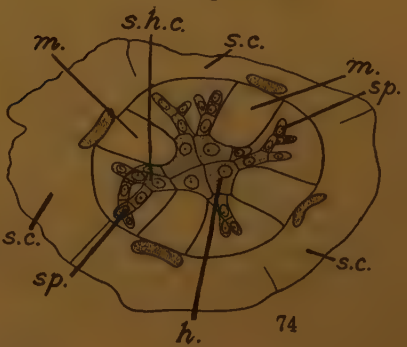

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TEXT-FIGS. 63–74

TEXT-FIGS. 63–74. *Chara zeylanica*. Fig. 63. L.S. of young antheridium (24-celled stage) and oogonium. Fig. 64. T.S. of branchlet node showing the antheridium (24-celled stage) and the two lateral bracteoles (*br.*). Figs. 65-68. T.L.S. of the node behind the antheridium showing stages in the development of the oogonial primordium, bracteoles and the downwardly growing cortical cells (*c.c.*). Figs. 69–73. Stages in the development of the antheridium. Fig. 74. Section of the antheridium showing early stages of development of spermatogenous filaments. *br.*, bracteoles; *c.c.*, cortical cell; *cr.*, crown cells; *h.*, head cell; *m.*, manubrium; *o.m.c.*, oogonial mother-cell; *o.p.*, oogonial primordium; *p.c.*, peripheral cell; *ped.*, pedicel cell. *s.c.*, shield cell; *s.h.c.*, secondary head-cell; *s.p.*, spermatogenous filament. (Figs. 63, 64, × 325; Figs. 65–73, × 450; Fig. 74, × 180.)

DEVELOPMENT OF THE ANTHERIDIUM AND THE OOGONIUM

Braun (1852) and Sachs (1882) were the first workers to give a detailed account of the development of the antheridium and the oogonium. Since then not much further work appears to have been done on the developmental morphology of these structures in *Chara*.

Chara zeylanica is monœcious. The antheridia and oogonia are generally produced at the lower most four free nodes of the branchlet on its inner side, *i.e.*, on the side facing the main axis. And since there is no torsion in the branchlet, the successive antheridia and oogonia are found disposed in a vertical line one above the other. As the upper portion of the branchlet developes earlier, the sexual organs formed in the upper nodes are older than those formed at the lower nodes.

The oldest peripheral cell of the branchlet node, *i.e.*, the peripheral cell which is situated on the side facing the main stem, gives rise to the antheridial and the oogonial primordia (Text-Figs. 52–58). This cell divides into an outer and an inner cell (Text-Fig. 53). The inner cell divides again by a wall parallel to the previous one into two cells, so that a row of three cells is formed (Text-Figs. 53, 54, 56). The outermost cell is the antheridial primordium (Text-Figs. 54, 57 *a.p.*) while the middle cell is of the nature of a node which by tangential walls cuts off five peripheral cells round a central cell (Text-Figs. 65–68). Of these five cells, the uppermost cell (adaxial to the antheridial primordium) enlarges and forms the oogonial primordium (Text-Figs. 58, 65–67, *o.p.*) the second and the third cells, (*i.e.*, the cells on either flank of the nodal cell) grow out into elongate one-celled bracteoles (Text-Figs. 64, 68, *br.*) and the fourth and the fifth cells give rise to the downwardly growing cortical rows of the branchlet (Text-Figs. 61, 62, 68, *c.c.*).

Thus the antheridium occupies the place of a bract cell and is homologous with it. *The antheridium is thus a metamorphosed terminal segment of a branch of the second order and the oogonium represents a metamorphosed branch of the third order.*

Development of the Antheridium

The antheridial primordium cuts off by a vertical wall a discoid cell at the base (*pedicel cell*) and a terminal cell, which soon assumes a spherical shape (Text-Fig. 60). This globular cell is the mother cell of the antheridium. It divides by a vertical wall into two hemispherical

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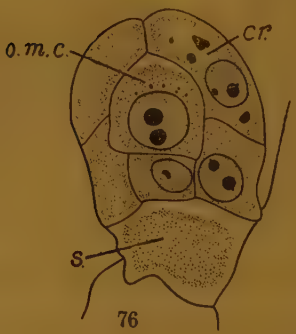

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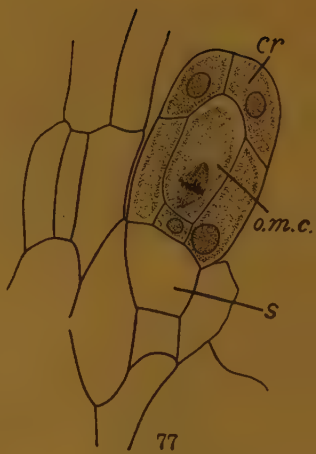

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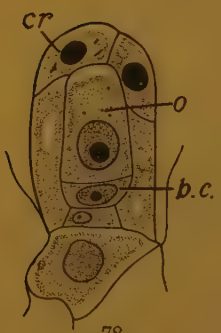

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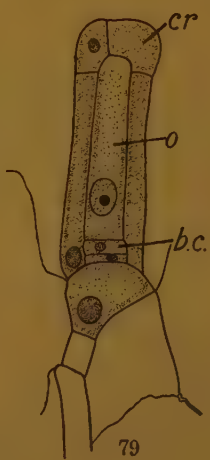

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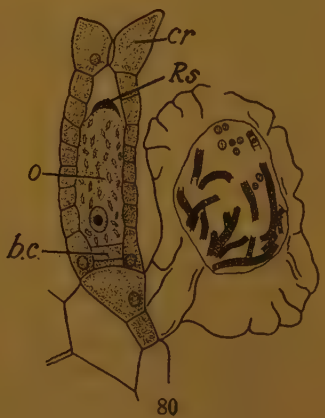

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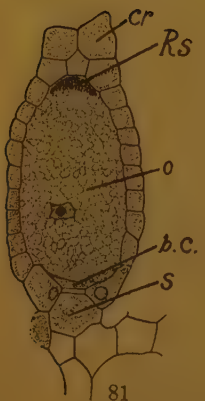

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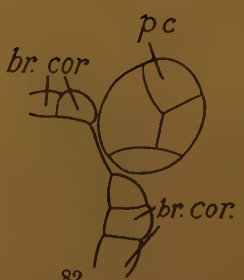

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TEXT-FIGS. 75–83

TEXT-FIGS. 75–83. *Chara zeylanica.* Figs. 75–81. L.S. of the young oogonium showing stages of development (L.S. of the antheridium with spermatogenous filaments also shown in Fig. 80). Fig. 82, 83. T.S. of the branchlet and the developing oogonium (T.S.) alongside. Note the gap due to the absence of cortical cells on the side close to the oogonium in Fig. 82, and how the cortical cells close in above in Fig. 83. *b.c.*, basal cell; *br. cor.*, branchlet cortex; *c.c.*, central cell; *cr.*, crown cell; *o.*, egg-cell; *o.m.c.*; oogonial mother-cell; *p.c.*, peripheral cell; *R.S.*, receptive spot; *s.*, stalk cell. (Figs. 75, 76, × 463; Figs. 77, 78, 82, 83, × 334; Fig. 79, × 220; Figs. 80, 81, × 90.)

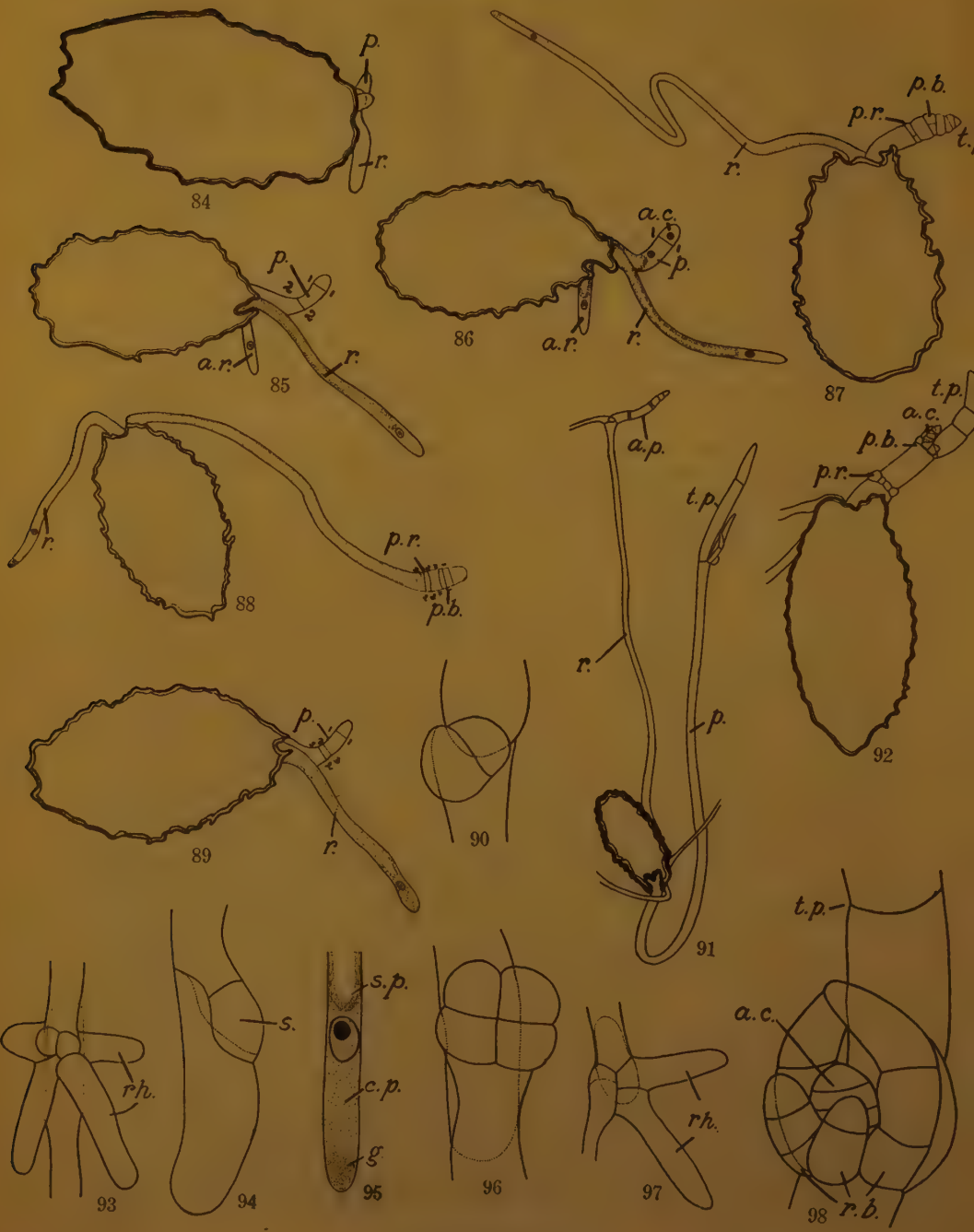
cells (Text-Fig. 69) each of which divides again by another vertical wall at right angles to the previous one. Each of the four quadrants thus formed divides transversely into two cells and eight cells are formed (Text-Fig. 69). Each octant divides periclinally into an outer and an inner cell (Text-Fig. 70). The outer cell again divides periclinally (Pl. XI, Fig. 1; Text-Fig. 71). According to Sachs (1882) and Migula (1897), the inner-cell divides periclinally. In the present *Chara*, the writer finds that the outer cell divides periclinally and not the inner cell. Eight radial series of three cells each are thus formed (Pl. XI, Fig. 2; Text-Fig. 72).

Now in each octant the three cells formed grow unequally so that they gradually become separated from one another and a cavity develops within the antheridium (Pl. XI, Fig. 6; Text-Fig. 74). By this time the basal cell (*pedicel cell*) protrudes into the cavity of the antheridium. The eight outer cells expand laterally and form the *shield cells* of the antheridium. The outer walls of these shield cells form radial ingrowths (which however do not reach the inner wall of the cell) as a result of which the cell, in a longitudinal section, appears as though divided into a number of compartments. The middle segment in each octant elongates radially thus becoming cylindrical and forms the *manubrium* (Pl. XI, Fig. 6; Text-Fig. 74, *m.*). The innermost segment becomes rounded off and forms the *capitulum* (head cell) (Pl. XI, Fig. 6; Text-Fig. 74, *h.*). Each capitulum cuts off on its inner side 2–6 cells, the *secondary capitula* (secondary head cells) (Text-Fig. 74, *s.h.c.*). The secondary capitula are in contact with each other and by further divisions give rise to the spermatogenous filaments (Pl. XI, Fig. 6; Text-Fig. 74, *sp.*). Each secondary capitulum gives rise to 2 or 3 filaments. Each spermatogenous filament has about 60-75 discoid cells which are in the early stages about as broad as long. The long spermatogenous filaments remain coiled inside the antheridium.

The development of the spermatogenous cells and spermatogenesis are given in detail elsewhere (Sundaralingam, 1946).

Development of the Oogonium

The primordium of the oogonium divides by a transverse wall into an upper apical cell, which becomes the oogonial mother cell, and a segment which divides again into two cells by a wall parallel to the first division (Text-Figs. 59, 60). The lower one of these two cells which corresponds to the first internode of a branch does not divide any further, but forms the stalk cell of the oogonium (Text-Figs. 75–77, *s*). The upper cell is of the nature of a nodal cell and divides by



TEXT-FIGS. 84–98

TEXT-FIGS. 84–98. *Chara zeylanica*. Figs. 84–89, 92. Stages in the germination of the oospore and the formation of the protonema. Figs. 90, 93, 94, 96, 97. Stages in the formation of the rhizoids. Fig. 91. Development of an accessory protonema (*a.p.*) from the primary root. Fig. 95. Tip of the primary root showing the large nucleus and the streaming protoplasm (*s.p.*). Fig. 98. Protonemal branchlet node showing the apical cell (*a.c.*) of the adult plant. *a.c.*, apical cell; *a.p.*, accessory protonema; *a.r.*, accessory root; *c.p.*, compact protoplasm; *g.*, granules; *p.*, protonema; *p.b.*, protonemal branchlet node; *p.r.*, protonemal root node; *r.*, primary root; *r.h.*, rhizoid; *S.*, lens-shaped cell formed during rhizoid formation; *s.p.*, streaming protoplasm; *t.p.*, terminal process. (Fig. 84, × 53; Figs. 85–89, 92, × 37; Figs. 90, 95, 98, × 150; Fig. 91, × 17; Figs. 94, 96, × 220; Figs. 93, 97, × 165).

vertical walls into a circle of five peripheral cells round a central cell (Text-Figs. 61, 82, 83, *p.c.*). These five peripheral cells elongate upwards and envelop the oogonial mother cell (Pl. XI, Figs. 3, 5; Text-Figs. 75, 76). These five periphereal cells correspond to branches of the fourth order.

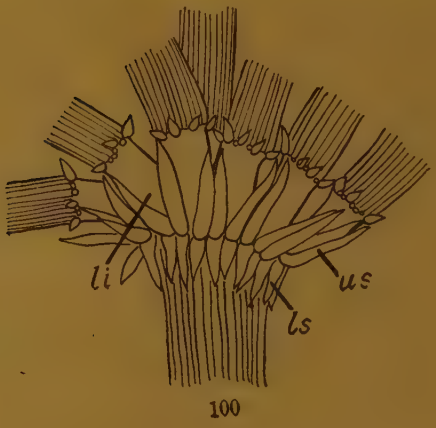
The oogonial mother-cell, after growing further, cuts off a narrow basal cell by a transverse wall (Pl. XI, Fig. 4; Text-Fig. 77) The upper cell becomes the egg cell. At a very early stage, each of the five enveloping cells divides into two cells by a transverse wall (Text-Fig. 76, *c.*). The upper cells remain short and form the crown cells, while the lower cells elongate very much and coil spirally round the elongated egg-cell, the spiral ascending from right to left (Text-Figs. 77–81, 102).

The egg-cell is at first completely filled with homogeneous cytoplasm (Text-Fig. 79). Soon starch grains make their appearance in large numbers (Text-Fig. 80). These starch grains are elongate, elliptical and narrow (Text-Fig. 80). But in the older mature oogonium the starch grains become much larger and are densely packed, with the result that the nucleus becomes much compressed and somewhat angular in appearance (Text-Fig. 81). In the young oogonium the nucleus is situated at the base of the oogonium and in the mature oogonium it assumes a more central position in the egg-cell (Text-Fig. 81). As the oogonium enlarges, the basal cell becomes very much compressed and could be recognised only as a narrow strip at the bottom of the egg cell (Text-Fig. 81, *b.c.*). In the mature oogonium the egg-cell is densely filled with starch grains except for a small portion at the apex of the cell where the protoplasm is finely granular and hyaline and forms the receptive spot (Text-Figs. 80, 81, *R.S.*).

On the side of the branchlet where an oogonium is formed no upward cortical system is developed, since the oogonium takes the place of a cortical cell. However, the adjacent cortical cells close in and fill up the gap (Text-Fig. 83). The gap could be seen, however, in a transverse section of the branchlet close to the base of the oogonium (Text-Fig. 82).

FERTILISATION

When the oogonium is mature the spiral enveloping cells, by an additional growth, lengthen slightly and form small gaps between the cells at the neck portion. The spermatozoids evidently pass through these gaps. Spermatozoids were found swarming near the mature oogonium, but their actual entry could not be observed.



TEXT-FIGS. 99–105

TEXT-FIGS. 99–105. *Chara zeylanica*. Fig. 99. Habit. Fig. 100. Stem node showing the two rows of stipulodes and the ecorticate lowermost internodes of the branchlets. Fig. 101. Stem cortex showing its triplostichous nature and the spine cells. Fig. 102. Lowermost fertile node of the branchlet with the sex organs. Figs. 103–105. Lowermost sterile nodes of the branchlets. Note the ecorticate lowermost internode. *br*, bracteole; *li*, lowermost internode of branchlet; *ls*, lower stipulode; *ps*, primary cortical series; *s*, spine cell; *ss*, secondary cortical series; *us*, upper stipulode. (Fig. 99, × ⅔; Figs. 100, 103–105, × 17; Figs. 101, 102, × 20).

Germination

Only a few workers have studied the germination of the oospore of the Characeæ. Pringsheim (1862) studied the germination of the oospore in *Ch. fragilis* and elucidated the structure of the proembryo, which was not clearly understood till then, though only just touched upon by some of the earlier authors. De Bary (1875) made a study of the germination of the oospore in *Chara fætida, Ch. fragilis, Ch. contraria, Ch. crinita, Ch. scoparia, Tolypella intricata, T. glomerata, Nitella capitata, N. tenuissima* and *N. hyalina.*

No work has been done so far in India on the germination of any of the Characeæ.

Ripe oospores of *Chara zeylanica* were collected from a tank in Pamban in South India along with the silt found at the bottom of the tank and brought to Madras and kept in the laboratory, in a well-lighted place close to the window in a tall glass jar, in freshly collected rain water. After about a fortnight, a few green germlings were observed growing above the silt. Since the oospores were in the soil, the early stages of germination could not be followed. So a number of still ungerminated oospores were sifted from the silt and kept in separate glass culture jars in freshly collected rain water. After about 48 hours, the oospores showed signs of germination.

The commencement of germination is indicated by the oospore wall cracking open into teeth-like structures at the anterior end. After 24 hours, two small knob-like structures appear at the anterior end of the oospore (Text-Fig. 84). The origin of these structures could not be seen clearly as the oospore wall is very opaque and thick. A number of germinating oospores were carefully dissected under a Greenough binocular microscope with the help of fine needles, and some stages of germination were followed.

At first the starch grains and other reserve food materials appear to recede from the apical portion and soon the oospore divides by a transverse wall into a small upper plano-convex cell filled with granular protoplasm and a larger lower cell densely packed with large starch grains. The upper cell again divides by a vertical wall into two cells, each of which grows out into a knob-like structure. The next day the two knob-like structures grow longer into tubular structures, one of which takes an upward direction (*the protonema*) (Text-Figs. 84–86, *p.*) and the other an opposite direction (*the primary root*) (Text-Fig. 84, *r.*). The primary root elongates very rapidly whereas the growth of the protonema is slow. At the extreme terminal portion of the protonema and of the primary root, the protoplasm is quite compact without any vacuole and does not show any movement (Text-Fig. 95). The single nucleus of the protonema and the primary root is embeded in this compact cytoplasm at the tip. The streaming of protoplasm could be seen immediately behind the nucleus. At the extreme tip a number of refractive granules could be seen in the compact protoplasm (Text-Fig. 95, *g.*). These are probably the bodies which are interpreted by Giesenhagen (1901) and Schröder (1904) as statoliths. The streaming

protoplasm carries along with it the large starch grains. In rare cases the protoplasmic streaming extends up to the extreme tip, and in such cases, the nucleus is found at the side close to the cell wall.

The tubular structure forming the protonema slightly enlarges in diameter. At the upper portion of the region of compact protoplasm in the protonema an apical cell is cut off by a transverse wall (Text-Fig. 86, 1–1, *a.c*). This apical cell is of limited growth and cuts off, at a later stage, 3–5 cells below in a row (Text-Figs. 87, 92). These cells at the apex of the protonema form the terminal process of the protonema (Text-Figs. 87, 92, *t.p.*). The portion of the protonema just below this terminal process enlarges a little and a transverse wall separating this swollen portion from the elongated lower portion of the protonema is then formed (Text-Fig. 85, 2–2). Then two transverse walls are formed in quick succession in this swollen portion between the two transverse walls already formed (Text-Figs. 88, 89, 3–3, 4–4). This results in a lower and an upper discoid cell, which are of the nature of nodal cells, separated by a cylindrical intermediate cell, which elongates and forms an internodal cell (Text-Figs. 87, 92). Of the two nodes of the protonema, the upper one is the protonemal branchlet node and the lower one is the protonemal root node (Text-Figs. 87, 92, *p.b.*, *p.r.*). The root-node, by a series of irregular divisions, forms a complex group of cells consisting of a few central cells surrounded by a number of peripheral cells, which later on grow out into rhizoids. The protonemal branchlet nodal cell is from an early stage thicker on one side than on the other (Pl. XI, Fig. 7; Text-Fig. 87). This difference in the thickness becomes more pronounced with further divisions formed in the protonemal branchlet node (Text-Fig. 92). The first formed cells, as a rule, are larger than the later-formed ones (Text-Fig. 92). The first formed cell is the initial cell of the adult plant. The other peripheral cells after a few transverse divisions develop into incurved rudimentary branchlets (Text-Fig. 98, *r.b.*). The terminal process is later on bent to one side by the stronger growth of the adult plant initial cell (Text-Fig. 92).

The adult plant initial cell divides by an oblique tangential wall into two cells of which the outer one protrudes from the node and becomes the apical cell of the adult plant (Text-Fig. 92, *a.c.*). This apical cell cuts off transverse segments continuously below as in the case of the main stem (Text-Fig. 98, *a.c.*). The first segment of this apical cell forms the first node of the new *Chara* plant.

The lowermost internode of the protonema elongates very much, the amount of elongation depending upon how deep the oospore is buried in the soil (Text-Fig. 91, *p.*). This internode is very short in the case of germlings grown in culture jars without any silt but is fairly long in cases where the oospores are buried in a certain amount of silt.

Calcium incrustations soon begin to be secreted on the shoot portions of the young germlings but no calcium incrustations are secreted on the root portions. The germlings grow better in a substratum of soft mud than in liquid cultures. They are strongly

positively-phototropic. The apical portion of the main axis of the young plants grows bent towards the source of light.

The roots form nodes from which long cylindrical rhizoids are formed. At first a curved oblique septum is formed dividing the root into two portions (Text-Fig. 94). The lower end of one portion becomes swollen and is cut off by an oblique wall (Text-Fig. 94). The newly formed cell by two divisions, one longitudinal and the other transverse, forms four cells (Text-Figs. 90, 96). Each of these cells develops into a rhizoid (Text-Figs. 93, 97, *rh.*). Thus a large number of rhizoids is formed from the several nodes formed in the primary and accessory roots of the protonema, with slight variations in the mode of their origin.

Accessory protonemas were found growing from (1) the rhizoid node, and (2) from a primary root (Text-Fig. 91, *a.p.*).

The first internodes of the germlings are ecorticate (Text-Fig. 91) The first internode of the adult plant starting from the axil of the rudimentary branchlets of the germling is, however, clothed with a cortex. In the first free node of the adult plant the two rows of stipulodes are imperfectly developed. Some of the branchlets starting from the first free node are ecorticate and very short. Only from the second free node of the adult plant are the two rows of stipulodes well formed. The branchlets formed from the second node onwards resemble the normal branchlets. As in the normal branchlets, the lowermost segments are ecorticate and the upper segments corticate.

Summary

The structure and development of the stem, the branchlets, the branch, cortex and stipulodes were followed in detail.

The development of the antheridium and of the oogonium was followed in full detail.

Oospores were germinated in the laboratory and all the stages of germination recorded. The details of germination observed were very similar to those recorded by De Bary in other species of *Chara*.

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EXPLANATION OF PLATES *Chara zeylanica*

PLATE X

FIGS. 1–7. Fig. 1. L.S. of the stem apex showing the first segment of the young branchlet dividing to form the basal nodal cell and the primary internodal cell, × 380. Fig. 2. L.S. of the stem apex showing the apical cell cutting off a transverse segment below, × 380. Fig. 3. L.S. of the stem apex showing the dome-shaped apical cell, × 175. Fig. 4. L.S. of the apex of the young branch in the axil of the oldest branchlet. Note the apical cell undergoing division, × 238. Fig. 5. Tangential L.S. of the young stem cortex. Note the segmentations of the downwardly growing and the upwardly growing cortical lobes and also their dovetailing with each other, × 158. Fig. 6. T.S. of the branchlet nodes showing the peripheral cells undergoing periclinal divisions. Note also alongside the T.S. of the node of the main axis with their basal nodes dividing to form the stipulodes, × 284. Fig. 7. L.S. of the branchlet showing the bract and the upwardly and downwardly growing cortical cells at each node, × 98.

PLATE XI

FIGS. 1–8. Fig. 1. T.L. section of the young antheridium (16-celled stage) showing eight cells, of which the four outer ones are undergoing division to form the shield cells and the manubria, × 742. Fig. 2. T.L. section of the young antheridium at a slightly later stage (24-celled stage) showing 4 radial rows of 3

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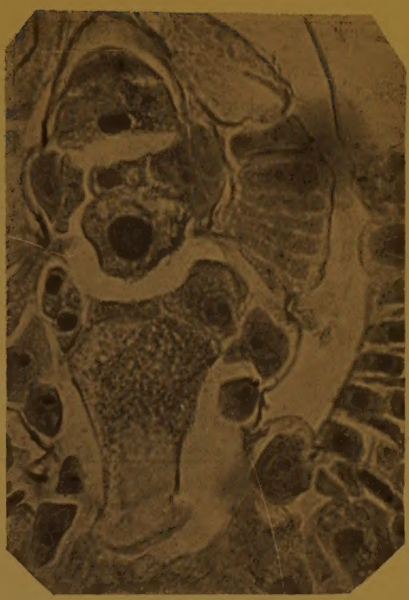

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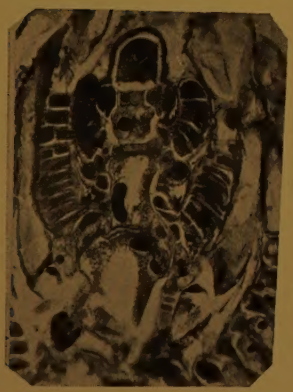

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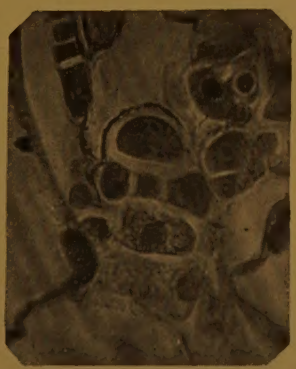

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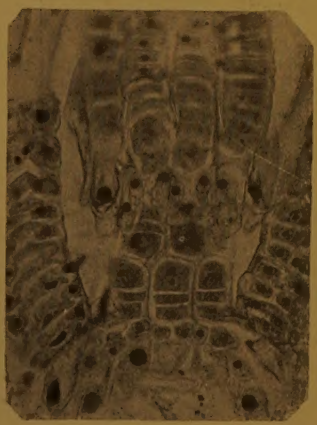

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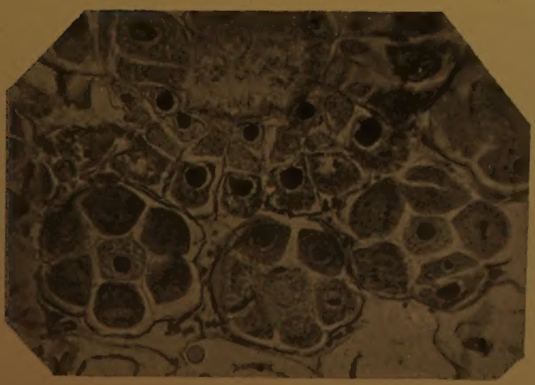

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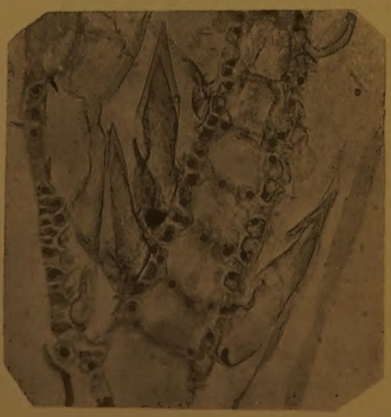

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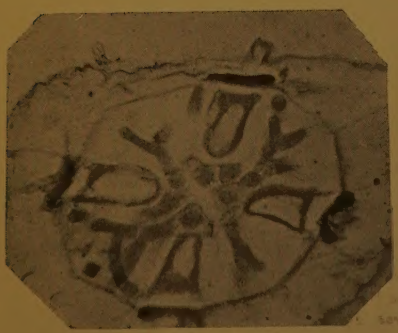

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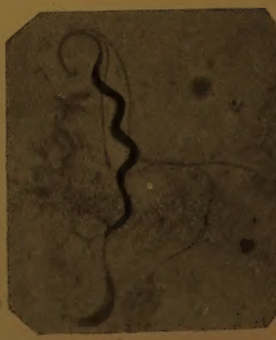

8

cells each, × 742. Fig. 3. L.S. of the young oogonium showing the oogonial mother-cell and two of the peripheral cells of the node below elongating upwards, × 595. Fig. 4. L.S. of the young oogonium at a later stage showing the oogonial mother cell dividing to form the basal cell. Note also the crown cells, × 424. Fig. 5. L.S. of the young oogonium showing two of the elongating peripheral cells with crown cells cut off at their apices, × 742. Fig. 6. T.L.S. of the antheridium showing the shield cells, manubria and the head-cells. Note the head-cells forming the spermatogenous filaments, × 476. Fig. 7. Germination of the oospore showing the protonema and the primary root, × 29. Fig. 8. A liberated spermatozoid showing the spirally coiled body and a pair of cilia inserted a little behind the extreme tip, × 952.

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